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(54) Title: METHODS FOR REVERSIBLY INHIBITING MYELOPOIESIS IN MAMMALIAN TISSUE

(57) Abstract

The present invention provides for the novel use of compounds of Formula (I) as myeloprotectant compounds having the biological activity of reversibly inhibiting myelopoiesis in mammalian tissue and biological samples, *in vitro*, *ex vivo* or *in vivo*.

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METHODS FOR REVERSIBLY INHIBITING MYELOPOIESIS IN MAMMALIAN TISSUE

FIELD OF THE INVENTION

The present invention relates to the field of myelopoiesis in mammalian tissue, and more particularly, to the use of certain imidazoles containing compounds for reversibly inhibiting myelopoiesis in humans and animals.

BACKGROUND OF THE INVENTION

Certain chemotherapeutic and radiation treatments for cancer and other serious illness have the undesired side effect of killing all dividing and differentiating cells. This effect is most severe on the differentiating cells of the hematopoietic system, which are essential for repopulating the bone marrow and permitting the redevelopment of a functioning immune system following such treatment. Normally, bone marrow toxicity or myelosuppression is the limiting factor in the use of such treatments. The acute effects of myelosuppression are the loss of neutrophils (neutropenia) and the loss of platelets (thrombocytopenia) in the blood. These conditions in part increase patient susceptibility to infections and hemorrhagic complications.

A variety of regulatory messengers and modifiers such as colony stimulating factors, interferons, and different types of compounds are responsible for the regulation of myelopoiesis, which is the development and growth of early progenitor cells (stem cells) of the hematopoietic system.

Compounds which have a selective inhibitory effect on the proliferation and differentiation of myelopoietic cells tend to prevent quiescent cells from entering into cell division and becoming susceptible to chemotherapeutic and radiation treatments. A number of such compounds are known, including MIP1α, HP5 monomer, TGFß, TNF and IL-1 (for radioprotection only) and AS101. See, e.g., International Patent Application No. WO9313789, published July 22, 1993, and

references cited therein. However, to date, there are no effective pharmaceutical compounds which protect a patient from leukopenia after treatments which create an insult to proliferating stem cells.

There remains a need for pharmaceutically acceptable compounds which are capable of serving as myeloprotectants, i.e., which can inhibit proliferation of normal hematopoietic stem cells during a period of myelosuppressive therapy.

SUMMARY OF THE INVENTION

The present invention provides for a method for reversibly inhibiting myelopoiesis in mammals, mammalian tissue or other samples *in vivo* and *ex vivo* comprising administering to said animal, tissue, or sample an effective amount of a myeloprotectant compound of formula (I) below. The amount of the compound is effective to reversibly inhibit the formation of myelopoietic colonies during the time the animal, tissue, or sample is exposed to myelosuppressive therapy.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 demonstrates that compounds of Formula (I), Compounds 2 and 3 are myeloprotective in a high dose 5-FU in vivo model; more specifically this is the CFU-GM Kinetics Post Compounds and 5-FU administration; 4-(4-Fluorophenyl)-5-(2-phenylamino-4-pyridiminyl)-1-(4-piperidinyl)imidazole is Compound 2 and 4-(4-Fluorophenyl)-5-(2-N-methylamino-4-pyridiminyl)-1-(4-N-methyl-piperidinyl)imidazole is Compound 3.

Figure 2 demonstrates a dose response effect of (IP administered) Compounds 2 and 3 on WBC counts 9 days post 5-FU in the mouse; *P<0.05.

Figure 3 demonstrates a dose response effect of (IP administered) Compounds 2 and 3 on PMN counts 9 days post 5-FU in the mouse; *P<0.05.

Figure 4. demonstrates a dose response effect of (IP administered) Compounds 2 and 3 on Platelet counts 9 days post 5-FU in the mouse; *P<0.05.

Figure 5 demonstrates a dose response effect of (IP administered) Compounds 2 and 3 on lymphocyte counts 9 days post 5-FU in the mouse; *P<0.05.

Figure 6 demonstrates a dose response effect of (IP administered) Compounds 2 and 3 on monocyte counts 9 days post 5-FU in the mouse; *P<0.05.

Figure 7 demonstrates a dose response effect of (IP administered) Compounds 2 and 3 on RBC counts 9 days post 5-FU in the mouse; *P<0.05.

Figure 8 demonstrates a dose response effect of (IP administered) Compounds 2 and 3 on bone marrow cell counts 9 days post 5-FU in the mouse. Marrow counts obtained from pools of 3 mice/group.

Figure 9 demonstrates a dose response effect of (PO administered) Compounds 2 and 3 on PMN cell counts 9 days post 5-FU in the mouse.

Figure 10 demonstrates a dose response effect of (PO administered) Compounds 2 and 3 on bone marrow cell counts 9 days post 5-FU in the mouse. Marrow counts obtained from pools of 3 mice/group.

Figure 11 demonstrates the effect of Compounds 2 and 3 and G-CSF on the kinetics of neutrophil recovery post 175 mg/kg 5-FU in mice. G-CSF (50ug/kg) administered daily IP, animals were bleed 1.5 hours post G-CSF injection on the indicated days.

Figure 12 demonstrates the effect of Compounds 2 and 3, and G-CSF on the kinetics of monocyte recovery post 175 mg/kg 5-FU in mice. G-CSF (50ug/kg) administered daily IP, animals were bleed 1.5 hours post G-CSF injection on the indicated days.

Figure 13 demonstrates the effect of Compounds 2 and 3, and G-CSF on the kinetics of platelet recovery post 175 mg/kg 5-FU in mice. G-CSF (50ug/kg) administered daily IP, animals were bleed 1.5 hours post G-CSF injection on the indicated days.

Figure 14 demonstrates the effect of Compounds 2 and 3, and G-CSF on the kinetics of lymphocyte recovery post 175 mg/kg 5-FU in mice. G-CSF (50ug/kg) administered daily IP, animals were bleed 1.5 hours post G-CSF injection on the indicated days.

Figure 15 demonstrates the effect of Compounds 2 and 3, and G-CSF on the kinetics of total white blood cell recovery post 175 mg/kg 5-FU in mice. G-CSF

(50ug/kg) administered daily IP, animals were bleed 1.5 hours post G-CSF injection on the indicated days.

Figure 16 demonstrates the effect of Compounds 2 and 3, and G-CSF on the kinetics of red blood cell recovery post 175 mg/kg 5-FU in mice. G-CSF (50ug/kg) administered daily IP, animals were bleed 1.5 hours post G-CSF injection on the indicated days.

Figure 17 demonstrates the effect of Compound 3, G-CSF, and the combination of Compound 3 + G-CSF on the kinetics of neutrophil recovery post 175 mg/kg 5-FU in mice. G-CSF (50ug/kg) administered daily IP, animals were bleed 1.5 hours post G-CSF injection on the indicated days.

Figure 18 demonstrates the effect of Compound 3, G-CSF, and the combination of Compound 3 + G-CSF on the kinetics of platelet recovery post 175 mg/kg 5-FU in mice. G-CSF (50ug/kg) administered daily IP, animals were bleed 1.5 hours post G-CSF injection on the indicated days.

Figure 19 demonstrates the effect of Compound 3, G-CSF, and the combination of Compound 3 + G-CSF on the kinetics of monocyte recovery post 175 mg/kg 5-FU in mice. G-CSF (50ug/kg) administered daily IP, animals were bleed 1.5 hours post G-CSF injection on the indicated days.

Figure 20 demonstrates the effect of Compound 3, G-CSF, and the combination of Compound 3 + G-CSF on the kinetics of total white blood cell recovery post 175 mg/kg 5-FU in mice. G-CSF (50ug/kg) administered daily IP, animals were bleed 1.5 hours post G-CSF injection on the indicated days.

Figure 21 demonstrates the CFU -GM Micro-Inhibition and Reversibility Assay for Select Compounds of Formula (I), wherein Compound 1 is 4-(4-Fluorophenyl)-5-(2-amino-4-pyridiminyl)-1-(1-methyl-4-piperidinyl)-imidazole, and Compound 2 is 4-(4-Fluorophenyl)-5-(2-phenylamino-4-pyridiminyl)-1-(4-piperidinyl)imidazole.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides for a method for reversibly inhibiting myelopoiesis in mammals, mammalian tissue, including bone marrow and other samples, *in vivo* and *ex vivo*. This method entails administering to the mammal, tissue or sample an amount of a compound of formula (I) effective to reversibly inhibit the formation of myelopoietic colonies during the time the mammal, tissue or bone marrow is exposed to myelosuppressive therapy. In this method, the myelopoietic colonies inhibited are CFU-C colony forming cells.

The myelopoietic CFU-C colony forming cells as used herein include, but are not limited to, CFU-G, CFU-M, CFU-GM, CFU-GEMM, CFU-Meg, and HPP. The terms are defined as CFU = colony forming unit, G = granulocyte, M= macrophage or megakaryocyte, E = erythroid colony types, HPP = high proliferative potential colony. A preferred colony forming cell is CFU-GM.

This method may be accomplished *in vivo*. In other words, a mammalian subject, preferably a human patient, undergoing chemotherapy or radiation may be administered the compound so that the reversible inhibition of myelopoiesis occurs *in vivo*.

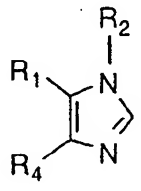
Alternatively, the method may be accomplished *ex vivo* or *in vitro*. According to this embodiment of the method, the mammalian tissue, for example, bone marrow, is contacted *ex vivo* with an effective amount of myeloprotectant compound of this invention substantially simultaneously with exposure of the tissue to radiation or chemotherapeutics. Inhibition of myelopoiesis is reversible when administration of the insult and the compound, which occur *ex vivo*, cease.

The myeloprotectant compounds, described herein, when administered prophylactically and/or therapeutically as a myelosuppressive treatment, benefit the recipient by protecting the normal hematopoietic stem cells from the damaging agent(s). Treatment with a myeloprotectant compound of this invention may be necessary throughout the time period in which the insulting agent is present within the body. Inhibition of normal hematopoietic stem cells during this period could protect normal cells while damaging only target cells such as cancer cells.

Because the compounds of this invention have demonstrated reversible inhibition, as demonstrated in the Example Section below, a more rapid rebound of hematopoietic stem cell proliferation is expected when administration of the myeloprotectant compound is ceased (concurrently with, or shortly after administration of the chemotherapeutic or radiation is ceased). The end result of treatment with the compounds of this invention as myeloprotective agents is to prevent neutropenia and or accelerate neutrophil / leukocyte recovery, and prevent stem cell loss due to myelosuppression. The advantage of such administration to the patient includes fewer infections due to leukopenia and the ability to use more aggressive chemotherapy regimens to kill cancer cells (dose intensification / increase dose frequency).

Compounds for use herein include the cytokine inhibitors as described in USSN 08/091,491, published as WO95/02575; WO96/21452; US Patent No.: ; USSN 08/473,396; US Patent No. 5,658,903 ; USSN 08/764,003; USSN 08/473,398; WO96/21654; WO93/14081; US Patent 5,656,644;USSN 08/095,234; US 5,656,644; WO95/03297; USSN 08/481,671; PCT/US97/00619; PCT/US97/00614; PCT/US97/00500; PCT/US97/00529; USSN 60/013,357; USSN 60/013,358; USSN 60/013,359; WO93/14082; WO95/13067;WO95/31451 WO95/13067;WO95/31451 WO 97/05877; WO 97/05878; WO 97/16441; WO 97/16426; and WO 97/16442. Each of these references are incorporated by reference herein in their entirety.

A preferred group of compounds for use herein are those compounds of the formula (I):



(I)

wherein:

R_1 is 4-pyridyl, pyrimidinyl, 4-pyridazinyl, 1,2,4-triazin-5-yl, quinolyl, isoquinolinyl, quinazolin-4-yl, 1-imidazolyl or 1-benzimidazolyl ring, which

ring is optionally substituted independently one to three times with Y, NHR_a, optionally substituted C_{1-4} alkyl, halogen, hydroxyl, optionally substituted C_{1-4} alkoxy, optionally substituted C_{1-4} alkylthio, C_{1-4} alkylsulfinyl, CH_2OR_{12}, amino, mono and di- C_{1-6} alkyl substituted amino, or $N(R_{10})C(O)R_b$;

Y is $O\text{-}R_a$;

R_4 is phenyl, naphth-1-yl or naphth-2-yl, or a heteroaryl, which is optionally substituted by one or two substituents, each of which is independently selected, and which, for a 4-phenyl, 4-naphth-1-yl, 5-naphth-2-yl or 6-naphth-2-yl substituent, is halogen, cyano, nitro, $C(Z)NR_7R_{17}$, $C(Z)OR_{16}$, $(CR_{10}R_{20})_vCOR_{12}$, SR_5, SOR_5, OR_{12}, halo-substituted-C_{1-4} alkyl, C_{1-4} alkyl, $ZC(Z)R_{12}$, $NR_{10}C(Z)R_{16}$, or $(CR_{10}R_{20})_vNR_{10}R_{20}$ and which, for other positions of substitution, is halogen, cyano, $C(Z)NR_{13}R_{14}$, $C(Z)OR_3$, $(CR_{10}R_{20})_{m''}COR_3$, $S(O)_mR_3$, OR_3, halo-substituted-C_{1-4} alkyl, C_{1-4} alkyl, $(CR_{10}R_{20})_{m''}NR_{10}C(Z)R_3$, $NR_{10}S(O)_{m'}R_8$, $NR_{10}S(O)_{m'}NR_7R_{17}$, $ZC(Z)R_3$ or $(CR_{10}R_{20})_{m''}NR_{13}R_{14}$;

v is 0, or an integer having a value of 1 or 2;

n is an integer having a value of 1 to 10;

n' is 0, or an integer having a value of 1 to 10;

m is 0, or the integer 1 or 2;

m' is an integer having a value of 1 or 2,

m" is 0, or an integer having a value of 1 to 5;

R_2 is hydrogen, $(CR_{10}R_{20})_{n'}OR_9$, heterocyclyl, heterocyclylC_{1-10} alkyl, C_{1-10}alkyl, halo-substituted C_{1-10} alkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, C_{3-7} cycloalkyl, C_{3-7}cycloalkylC_{1-10} alkyl, C_{5-7} cycloalkenyl, C_{5-7} cycloalkenyl C_{1-10}alkyl, aryl, arylC_{1-10} alkyl, heteroaryl, heteroarylC_{1-10}alkyl, $(CR_{10}R_{20})_nOR_{11}$, $(CR_{10}R_{20})_nS(O)_mR_{18}$, $(CR_{10}R_{20})_nNHS(O)_2R_{18}$, $(CR_{10}R_{20})_nNR_{13}R_{14}$, $(CR_{10}R_{20})_nNO_2$, $(CR_{10}R_{20})_nCN$, $(CR_{10}R_{20})_{n'}SO_2R_{18}$, $(CR_{10}R_{20})_nS(O)_{m'}NR_{13}R_{14}$, $(CR_{10}R_{20})_nC(Z)R_{11}$, $(CR_{10}R_{20})_nOC(Z)R_{11}$, $(CR_{10}R_{20})_nC(Z)OR_{11}$, $(CR_{10}R_{20})_nC(Z)NR_{13}R_{14}$, $(CR_{10}R_{20})_nC(Z)NR_{11}OR_9$, $(CR_{10}R_{20})_nNR_{10}C(Z)R_{11}$, $(CR_{10}R_{20})_nNR_{10}C(Z)NR_{13}R_{14}$, $(CR_{10}R_{20})_nN(OR_6)C(Z)NR_{13}R_{14}$, $(CR_{10}R_{20})_nN(OR_6)C(Z)R_{11}$, $(CR_{10}R_{20})_nC(=NOR_6)R_{11}$, $(CR_{10}R_{20})_nNR_{10}C(=NR_{19})NR_{13}R_{14}$, $(CR_{10}R_{20})_nOC(Z)NR_{13}R_{14}$, $(CR_{10}R_{20})_nNR_{10}C(Z)NR_{13}R_{14}$, $(CR_{10}R_{20})_nNR_{10}C(Z)OR_{10}$, 5-$(R_{18})$-1,2,4-oxadizaol-3-yl or 4-(R_{12})-5-$(R_{18}R_{19})$-4,5-dihydro-1,2,4-oxadiazol-3-yl; wherein

the aryl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroaryl, heteroaryl alkyl, heterocyclic and heterocyclic alkyl groups may be optionally substituted;

Z is oxygen or sulfur;

R_a is C_{1-6}alkyl, aryl, arylC_{1-6}alkyl, heterocyclic, heterocyclylC_{1-6} alkyl, heteroaryl, or heteroarylC_{1-6}alkyl, wherein each of these moieties may be optionally substituted;

R_b is hydrogen, C_{1-6} alkyl, C_{3-7} cycloalkyl, aryl, arylC_{1-4} alkyl, heteroaryl, heteroarylC_{1-4}alkyl, heterocyclyl, or heterocyclylC_{1-4} alkyl;

R_3 is heterocyclyl, heterocyclylC_{1-10} alkyl or R_8;

R_5 is hydrogen, C_{1-4} alkyl, C_{2-4} alkenyl, C_{2-4} alkynyl or NR_7R_{17}, excluding the moieties $-SR_5$ being $-SNR_7R_{17}$ and $-SOR_5$ being -SOH;

R_6 is hydrogen, a pharmaceutically acceptable cation, C_{1-10} alkyl, C_{3-7} cycloalkyl, aryl, arylC_{1-4} alkyl, heteroaryl, heteroarylC_{1-4} alkyl, heterocyclic, aroyl, or C_{1-10} alkanoyl;

R_7 and R_{17} is each independently selected from hydrogen or C_{1-4} alkyl or R_7 and R_{17} together with the nitrogen to which they are attached form a heterocyclic ring of 5 to 7 members which ring optionally contains an additional heteroatom selected from oxygen, sulfur or NR_{15};

R_8 is C_{1-10} alkyl, halo-substituted C_{1-10} alkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, C_{3-7} cycloalkyl, C_{5-7} cycloalkenyl, aryl, arylC_{1-10} alkyl, heteroaryl, heteroarylC_{1-10} alkyl, $(CR_{10}R_{20})_nOR_{11}$, $(CR_{10}R_{20})_nS(O)_mR_{18}$, $(CR_{10}R_{20})_nNHS(O)_2R_{18}$, $(CR_{10}R_{20})_nNR_{13}R_{14}$; wherein the aryl, arylalkyl, heteroaryl, heteroaryl alkyl may be optionally substituted;

R_9 is hydrogen, $C(Z)R_{11}$ or optionally substituted C_{1-10} alkyl, $S(O)_2R_{18}$, optionally substituted aryl or optionally substituted aryl-C_{1-4} alkyl;

R_{10} and R_{20} is each independently selected from hydrogen or C_{1-4} alkyl;

R_{11} is hydrogen, C_{1-10} alkyl, C_{3-7} cycloalkyl, heterocyclyl, heterocyclyl C_{1-10}alkyl, aryl, arylC_{1-10} alkyl, heteroaryl or heteroarylC_{1-10} alkyl;

R_{12} is hydrogen or R_{16};

R_{13} and R_{14} is each independently selected from hydrogen or optionally substituted C_{1-4} alkyl, optionally substituted aryl or optionally substituted aryl-C_{1-4} alkyl, or together with the nitrogen to which they are attached form a heterocyclic ring of 5 to 7 members which ring optionally contains an additional heteroatom selected from oxygen, sulfur or NR9;

R_{15} is R_{10} or C(Z)-C_{1-4} alkyl;

R_{16} is C_{1-4} alkyl, halo-substituted-C_{1-4} alkyl, or C_{3-7} cycloalkyl;

R_{18} is C_{1-10} alkyl, C_{3-7} cycloalkyl, heterocyclyl, aryl, arylalkyl, heterocyclyl, heterocyclyl-C_{1-10}alkyl, heteroaryl or heteroarylalkyl;
R_{19} is hydrogen, cyano, C_{1-4} alkyl, C_{3-7} cycloalkyl or aryl;
or a pharmaceutically acceptable salt thereof.

Suitably R_1 is a substituted 4-pyridyl or 4-pyrimindyl. More suitably R_1 is substituted by alkoxy, alkylthio, amino, methylamino, NHRa, or Y. A preferred ring placement of the R_1 substituent on the 4-pyridyl derivative is the 2-position, such as 2-methoxy-4-pyridyl. A preferred ring placement on the 4-pyrimidinyl ring is also at the 2-position, such as in 2-methoxy-pyrimidinyl.

When the substituent is Y, and R_a is aryl, it is preferably phenyl or naphthyl. When R_a is aryl alkyl, it is preferably benzyl or napthylmethyl. When R_a is heterocyclic or heterocyclic alkyl moiety, the heterocyclic portion is preferably pyrrolindinyl, piperidine, morpholino, tetrahydropyran, tetrahydrothiopyranyl, tetrahydrothipyran-sulfinyl, tetrahydrothio-pyransulfonyl, pyrrolindinyl, indole, or piperonyl. It is noted that the heterocyclic rings herein may contain unsaturation, such as in a tryptamine ring.

The aryl, heterocyclic and heteroaryl rings may be optionally substituted one or more times independently with halogen; C_{1-4} alkyl, such as methyl, ethyl, propyl, isopropyl, or t-butyl; halosubstituted alkyl, such as CF_3; hydroxy; hydroxy substituted C_{1-4} alkyl; C_{1-4} alkoxy, such as methoxy or ethoxy; $S(O)_m$alkyl and S(O)m aryl (wherein m is 0, 1, or 2); $C(O)OR_{11}$, such as $C(O)C_{1-4}$ alkyl or C(O)OH moieties; $C(O)R_{11}$; $OC(O)R_c$; O-(CH_2)s-O-, such as in a ketal or dioxyalkylene bridge; amino; mono- and di-C_{1-6} alkylsubstituted amino; $N(R_{10})C(O)R_b$; $C(O)NR_{10}R_{20}$; cyano, nitro, or an N-heterocyclyl ring which ring has from 5 to 7 members and optionally contains an additional heteroatom selected from oxygen, sulfur or NR_{15}; aryl, such as phenyl; an optionally substituted arylalkyl, such as benzyl or phenethyl; aryloxy, such as phenoxy; or arylalkyloxy such as benzyloxy.

R_c is optionally substituted C_{1-6} alkyl, C_{3-7} cycloalkyl, aryl, arylC_{1-4} alkyl, heteroaryl, heteroarylC_{1-4}alkyl, heterocyclyl, or heterocyclylC_{1-4} alkyl moieties.

Preferably, the R_a groups include C_{1-4} alkyl, benzyl, halosubstituted benzyl, napthylmethyl, phenyl, halosubstituted phenyl, aminocarbonylphenyl, alkylphenyl, cyanophenyl, alkylthiophenyl, hydroxyphenyl, alkoxyphenyl, morpholinopropyl, piperonyl, piperidin-4-yl, alkyl substituted piperidine, such as 1-methyl piperidine, or 2,2,6,6-tetramethylpiperidin-4-yl.

Preferably, when the substituent is NHR_a then R_a is aryl, arylalkyl, halosubstituted arylalkyl, halosubstituted aryl, heterocyclic alkyl, hydroxy alkyl, alkyl-1-piperidine-carboxylate, heterocyclic, alkyl substituted heterocyclic, halosubstituted heterocyclic, or aryl substituted heterocyclic. More specifically R_a is benzyl, halosubstituted benzyl, napthylmethyl, phenyl, halosubstituted phenyl, morpholinopropyl, 2-hydroxy ethyl, ethyl-1-piperidinecarboxylate, piperonyl, piperidin-4-yl, alkyl substituted piperidine, chlorotryptamine, and tetrathiohydropyranyl.

Preferably, when the substituent is a substituted C_{1-4} alkoxy or C_{1-4} alkylthio, the alkyl chain is substituted by halogen, such as fluorine, chlorine, bromine or iodine; hydroxy, such as hydroxyethoxy; C_{1-10} alkoxy, such as a methoxymethoxy, S(O)m alkyl, wherein m is 0, 1 or 2; amino, mono & di-substituted amino, such as in the NR_7R_{17} group, i.e. tert-butylaminoethoxy; or where the R_7R_{17} may together with the nitrogen to which they are attached cyclize to form a 5 to 7 membered ring which optionally includes an additional heteroatom selected from O/N/S; C_{1-10} alkyl, cycloalkyl, or cycloalkyl alkyl group, such as methyl, ethyl, propyl, isopropyl, t-butyl, etc. or cyclopropyl methyl; or halosubstituted C_{1-10} alkyl, such as CF_3. Preferably the R_1 substituents are tertbutylaminoethoxy, or hydroxyethoxy.

Suitably, R_4 is an optionally substituted phenyl. Preferably the phenyl is substituted one or more times independently by halogen, SR_5, $S(O)R_5$, OR_{12}, halo-substituted-C_{1-4} alkyl, or C_{1-4} alkyl.

Suitably, R_2 is selected from hydrogen, C_{1-10} alkyl, optionally substituted heterocyclyl, optionally substituted heterocyclylC_{1-10} alkyl, $(CR_{10}R_{20})_nNS(O)_2R_{18}$, $(CR_{10}R_{20})_nS(O)_mR_{18}$, aryl$C_{1-10}$ alkyl, $(CR_{10}R_{20})_nNR_{13}R_{14}$, optionally substituted C_{3-7}cycloalkyl, or optionally

substituted C_{3-7}cycloalkyl C_{1-10} alkyl. Preferably R_2 is morpholino propyl, piperidine, N-methylpiperidine, N-benzylpiperidine, 2,2,6,6-tetramethylpiperidine, 4-aminopiperidine, 4-amino-2,2,6,6-tetramethyl piperidine, 4-hydroxycyclohexyl, 4-methyl-4-hydroxy cyclohexyl, 4-pyrrolinindyl-cyclohexyl, 4-methyl-4-aminocyclohexyl, 4-methyl-4-acetamidocyclohexyl, 4-keto cyclohexyl, 4-oxiranyl, or 4-hydroxy-4-(1-propynyl)cyclohexyl.

Preferably R_2 is hydrogen, optionally substituted heterocyclyl ring, optionally substituted heterocyclylC_{1-10} alkyl, optionally substituted C_{1-10} alkyl, optionally substituted C_{3-7}cycloalkyl, optionally substituted C_{3-7}cycloalkyl C_{1-10} alkyl, $(CR_{10}R_{20})_nC(Z)OR_{11}$ group, $(CR_{10}R_{20})_nNR_{13}R_{14}$, $(CR_{10}R_{20})_nNHS(O)_2R_{18}$, $(CR_{10}R_{20})_nS(O)_mR_{18}$, optionally substituted aryl; optionally substituted arylC_{1-10} alkyl, $(CR_{10}R_{20})_nOR_{11}$, $(CR_{10}R_{20})_nC(Z)R_{11}$, or $(CR_{10}R_{20})_nC(=NOR_6)R_{11}$ group.

More preferably R_2 is an optionally substituted heterocyclyl ring, and optionally substituted heterocyclylC_{1-10} alkyl, optionally substituted aryl, $(CR_{10}R_{20})_nNR_{13}R_{14}$, $(CR_{10}R_{20})_nC(Z)OR_{11}$ group, optionally substituted C_{3-7}cycloalkyl, or an optionally substituted C_{3-7}cycloalkyl C_{1-10} alkyl.

When R_2 is an optionally substituted heterocyclyl, the ring is preferably a morpholino, pyrrolidinyl, or a piperidinyl group. When the ring is optionally substituted, the substituents may be directly attached to the free nitrogen, such as in the piperidinyl group or pyrrole ring, or on the ring itself. Preferably the ring is a piperidine or pyrrole, more preferably piperidine. The heterocyclyl ring may be optionally substituted one to four times independently by halogen; C_{1-4} alkyl; aryl, such as phenyl; aryl alkyl, such as benzyl - wherein the aryl or aryl alkyl moieties themselves may be optionally substituted (as in the definition section below); $C(O)OR_{11}$, such as the $C(O)C_{1-4}$ alkyl or C(O)OH moieties; C(O)H; $C(O)C_{1-4}$ alkyl, hydroxy substituted C_{1-4} alkyl, C_{1-4} alkoxy, $S(O)_mC_{1-4}$ alkyl (wherein m is 0, 1, or 2), $NR_{10}R_{20}$ (wherein R_{10} and R_{20} are independently hydrogen or C_{1-4}alkyl).

Preferably if the ring is a piperidine, the ring is attached to the imidazole at the 4-position, and the substituents are directly on the available nitrogen, i.e. a

1-Formyl-4-piperidine, 1-benzyl-4-piperidine, 1-methyl-4-piperidine, 1-ethoxycarbonyl-4-piperidine. If the ring is substituted by an alkyl group and the ring is attached in the 4-position, it is preferably substituted in the 2- or 6- position or both, such as 2,2,6,6-tetramethyl-4-piperidine. Similarly, if the ring is a pyrrole, the ring is attached to the imidazole at the 3-position, and the substituents are all directly on the available nitrogen.

When R_2 is an optionally substituted heterocyclyl C_{1-10} alkyl group, the ring is preferably a morpholino, pyrrolidinyl, or a piperidinyl group. Preferably this alkyl moiety is from 1 to 4, more preferably 3 or 4, and most preferably 3, such as in a propyl group. Preferred heterocyclic alkyl groups include but are not limited to, morpholino ethyl, morpholino propyl, pyrrolidinyl propyl, and piperidinyl propyl moieties. The heterocyclic ring herein is also optionally substituted in a similar manner to that indicated above for the direct attachment of the heterocyclyl.

When R_2 is an optionally substituted C_{3-7}cycloalkyl, or an optionally substituted C_{3-7}cycloalkyl C_{1-10} alkyl, the cycloalkyl group is preferably a C_4 or C_6 ring, most preferably a C_6 ring, which ring is optionally substituted. The cycloalkyl ring may be optionally substituted one to three times independently by halogen, such as fluorine, chlorine, bromine or iodine; hydroxy; C_{1-10} alkoxy, such as methoxy or ethoxy; $S(O)_m$ alkyl, wherein m is 0, 1, or 2, such as methyl thio, methylsulfinyl or methyl sulfonyl; $S(O)_m$ aryl; cyano, nitro, amino, mono & di-substituted amino, such as in the NR_7R_{17} group, wherein R_7 and R_{17} are as defined in Formula (I), or where the R_7R_{17} may cyclize together with the nitrogen to which they are attached to form a 5 to 7 membered ring which optionally includes an additional heteroatom selected from oxygen, sulfur or NR_{15} (and R_{15} is as defined for Formula (I)); $N(R_{10})C(O)X_1$ (and X_1 is C_{1-4} alkyl, aryl or arylC_{1-4}alkyl); $N(R_{10})C(O)$ aryl; C_{1-10} alkyl, such as methyl, ethyl, propyl, isopropyl, or t-butyl; optionally substituted alkyl wherein the substituents are halogen, (such as CF_3), hydroxy, nitro, cyano, amino, mono & di-substituted amino, such as in the NR_7R_{17} group, $S(O)_m$alkyl and $S(O)_m$ aryl, wherein m is 0, 1 or 2; optionally substituted alkylene, such as ethylene or propylene; optionally substituted alkyne, such as ethyne; $C(O)OR_{11}$ (wherein R_{11} is as defined in Formula (I)), such as the free acid

or methyl ester derivative; the group R_e; C(O)H; =O; =N-OR_{11}; N(H)-OH (or substituted alkyl or aryl derivatives thereof on the nitrogen or the oxime moiety); N(OR_d)-C(O)-$R_{6'}$; an optionally substituted aryl, such as phenyl; an optionally substituted arylC_{1-4}alkyl, such as benzyl of phenethyl; an optionally substituted heterocycle or heterocyclic C_{1-4}alkyl, and further these aryl, arylalkyl, heterocyclic, and heterocyclic alkyl moieties are optionally substituted one to two times by halogen, hydroxy, C_{1-10} alkoxy, $S(O)_m$ alkyl, cyano, nitro, amino, mono & di-substituted amino, such as in the NR_7R_{17} group, an alkyl, halosubstituted alkyl.

Suitably R_d is hydrogen, a pharmaceutically acceptable cation, aroyl or a C_{1-10} alkanoyl group.

Suitably R_e is a 1,3-dioxyalkylene group of the formula -O-$(CH_2)_s$-O-, wherein s is 1 to 3, preferably s is 2 yielding a 1,3-dioxyethylene moiety, or ketal functionality. Suitably $R_{6'}$ is $NR_{19'}R_{20'}$ alkyl $_{1-6}$; halosubstituted alkyl $_{1-6}$; hydroxy substituted alkyl $_{1-6}$; alkenyl $_{2-6}$; aryl or heteroaryl optionally substituted by halogen, alkyl $_{1-6}$, halosubstituted alkyl$_{1-6}$, hydroxyl, or alkoxy $_{1-6}$.

Suitably $R_{19'}$ is H or alkyl$_{1-6}$.

Suitably $R_{20'}$ is H, alkyl$_{1-6}$, aryl, benzyl, heteroaryl, alkyl substituted by halogen or hydroxyl, or phenyl substituted by a member selected from the group consisting of halo, cyano, alkyl$_{1-12}$, alkoxy $_{1-6}$, halosubstituted alkyl$_{1-6}$, alkylthio, alkylsulphonyl, or alkylsulfinyl; or $R_{19'}$ and $R_{20'}$ may together with the nitrogen to which they are attached form a ring having 5 to 7 members, which members may be optionally replaced by a heteroatom selected from oxygen, sulfur or nitrogen. The ring may be saturated or contain more than one unsaturated bond. Preferably $R_{6'}$ is $NR_{19'}R_{20'}$ and $R_{19'}$ and $R_{20'}$ are preferably hydrogen.

When the R_2 cycloalkyl moiety is substituted by NR_7R_{17} group, or NR_7R_{17} C_{1-10} alkyl group, and the R_7 and R_{17} are as defined in Formula (I), the substituent is preferably an amino, amino alkyl, or an optionally substituted pyrrolidinyl moiety.

Preferably, $R^{1'}$ and $R^{2'}$ are hydrogen, hydroxy, alkyl, substituted alkyl, optionally substituted alkyne, aryl, arylalkyl, NR_7R_{17}, and $N(R_{10})C(O)R_{11}$.

Suitably, alkyl is C_{1-4} alkyl, such as methyl, ethyl, or isopropyl; NR_7R_{17} and NR_7R_{17} alkyl, such as amino, methylamino, aminomethyl, aminoethyl; substituted

alkyl such as in cyanomethyl, cyanoethyl, nitroethyl, pyrrolidinyl; aryl such as in phenyl; arylalkyl, such as in benzyl; optionally substituted alkyne, such as ethyne or propynyl; or together $R^{1'}$ and $R^{2'}$ are a keto functionality.

Preferably R_2 is an optionally substituted heterocyclic, heterocylic C_{1-4} alkyl, a cycloalkyl or a cycloalkyl alkyl. More preferably R_2 is an optionally substituted C_4 or C_6 cycloalkyl, cyclopropyl methyl, morpholinyl butyl, morpholinyl propyl, morpholinyl ethyl, cyclohexyl substituted by methyl, phenyl, benzyl, amino, acetamide, aminomethyl, aminoethyl, cyanomethyl, cyanoethyl, hydroxy, nitroethyl, pyrrolidinyl, ethynyl, 1-propynyl, =O, O-$(CH_2)_2$O-, =NOR_{11}, wherein R_{11} is hydrogen, alkyl or aryl, NHOH, or N(OH)-C(O)-NH_2; or R_2 is morpholinyl propyl, aminopropyl, piperidinyl, N-benzyl-4-piperidinyl, N-methyl-4-piperidinyl, 2,2,6,6-tetramethypiperidinyl, substituted piperidine, such as 1-Formyl-4-piperidine, or a 1-ethoxycarbonyl-4-piperidine.

Preferred compounds of Formula (I) include:

4-(4-Fluorophenyl)-5-(2-amino-4-pyridiminyl)-1-(1-methyl-4-piperidinyl)imidazole;

4-(4-Fluorophenyl)-5-(2-phenylamino-4-pyridiminyl)-1-(4-piperidinyl)imidazole;

4-(4-Fluorophenyl)-5-(2-N-methylamino-4-pyridiminyl)-1-(4-N-methyl-piperidinyl)imidazole;

4-(4-Fluorophenyl)-5-(2-N-methylamino-4-pyridiminyl)-1-(4-piperidinyl)imidazole;or pharmaceutically acceptable salts thereof.

In all instances herein where there is an alkenyl or alkynyl moiety as a substituent group, the unsaturated linkage, i.e., the vinylene or acetylene linkage is preferably not directly attached to the nitrogen, oxygen or sulfur moieties, for instance in OR_3, or for certain R_2 moieties.

As used herein, "optionally substituted", unless specifically defined, shall mean such groups as halogen, such as fluorine, chlorine, bromine or iodine; hydroxy; hydroxy substituted C_{1-10}alkyl; C_{1-10} alkoxy, such as methoxy or ethoxy; S(O)m alkyl, wherein m is 0, 1 or 2, such as methyl thio, methylsulfinyl or methyl sulfonyl; amino, mono & di-substituted amino, such as in the NR_7R_{17} group; or

where the R_7R_{17} may together with the nitrogen to which they are attached cyclize to form a 5 to 7 membered ring which optionally includes an additional heteroatom selected from O/N/S; C_{1-10} alkyl, cycloalkyl, or cycloalkyl alkyl group, such as methyl, ethyl, propyl, isopropyl, t-butyl, etc. or cyclopropyl methyl; halosubstituted C_{1-10} alkyl, such CF_3; an optionally substituted aryl, such as phenyl, or an optionally substituted arylalkyl, such as benzyl or phenethyl, wherein these aryl moieties may also be substituted one to two times by halogen; hydroxy; hydroxy substituted alkyl; C_{1-10} alkoxy; $S(O)_m$ alkyl; amino, mono & di-substituted amino, such as in the NR_7R_{17} group; alkyl, or CF_3.

Suitable pharmaceutically acceptable salts are well known to those skilled in the art and include basic salts of inorganic and organic acids, such as hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, methane sulphonic acid, ethane sulphonic acid, acetic acid, malic acid, tartaric acid, citric acid, lactic acid, oxalic acid, succinic acid, fumaric acid, maleic acid, benzoic acid, salicylic acid, phenylacetic acid and mandelic acid. In addition, pharmaceutically acceptable salts of compounds of Formula (I) may also be formed with a pharmaceutically acceptable cation, for instance, if a substituent group comprises a carboxy moiety. Suitable pharmaceutically acceptable cations are well known to those skilled in the art and include alkaline, alkaline earth, ammonium and quaternary ammonium cations.

The following terms, as used herein, refer to:

- "halo" or "halogens", include the halogens: chloro, fluoro, bromo and iodo.
- "C_{1-10}alkyl" or "alkyl" - both straight and branched chain radicals of 1 to 10 carbon atoms, unless the chain length is otherwise limited, including, but not limited to, methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *sec*-butyl, *iso*-butyl, *tert*-butyl, *n*-pentyl and the like.
- The term "cycloalkyl" is used herein to mean cyclic radicals, preferably of 3 to 8 carbons, including but not limited to cyclopropyl, cyclopentyl, cyclohexyl, and the like.

• The term "cycloalkenyl" is used herein to mean cyclic radicals, preferably of 5 to 8 carbons, which have at least one bond including but not limited to cyclopentenyl, cyclohexenyl, and the like.

• The term "alkenyl" is used herein at all occurrences to mean straight or branched chain radical of 2-10 carbon atoms, unless the chain length is limited thereto, including, but not limited to ethenyl, 1-propenyl, 2-propenyl, 2-methyl-1-propenyl, 1-butenyl, 2-butenyl and the like.

• "aryl" - phenyl and naphthyl;

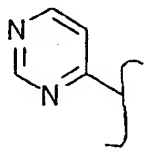
• "heteroaryl" (on its own or in any combination, such as "heteroaryloxy", or "heteroaryl alkyl") - a 5-10 membered aromatic ring system in which one or more rings contain one or more heteroatoms selected from the group consisting of N, O or S, such as, but not limited, to pyrrole, pyrazole, furan, thiophene, quinoline, isoquinoline, quinazolinyl, pyridine, pyrimidine, oxazole, thiazole, thiadiazole, triazole, imidazole, or benzimidazole.

• "heterocyclic" (on its own or in any combination, such as "heterocyclylalkyl") - a saturated or partially unsaturated 4-10 membered ring system in which one or more rings contain one or more heteroatoms selected from the group consisting of N, O, or S; such as, but not limited to, pyrrolidine, piperidine, piperazine, morpholine, tetrahydro pyran, or imidazolidine.

• The term "aralkyl" or "heteroarylalkyl" or "heterocyclicalkyl" is used herein to mean C_{1-4} alkyl as defined above attached to an aryl, heteroaryl or heterocyclic moiety as also defined herein unless otherwise indicated.

• "sulfinyl" - the oxide S (O) of the corresponding sulfide, the term "thio" refers to the sulfide, and the term "sulfonyl" refers to the fully oxidized $S(O)_2$ moiety.

For the purposes herein the "core" 4-pyrimidinyl moiety for R_1 or R_2 is referred to as the formula:



It is recognized that the compounds of the present invention may exist as stereoisomers, regioisomers, or diastereiomers. These compounds may contain one or more asymmetric carbon atoms and may exist in racemic and optically active forms. All of these compounds are included within the scope of the present invention.

As noted previously, methods of making these compounds can be found in their respective patent applications as noted above.

Compounds of Formula (I) are capable of inhibiting proinflammatory cytokines, such as IL-1, IL-6, IL-8 and TNF.

A member of the MAP kinase family, alternatively termed CSBP, p38, or RK, has been identified independently by several laboratories under different names. Activation of this novel protein kinase via dual phosphorylation has been observed in different cell systems upon stimulation by a wide spectrum of stimuli, such as physicochemical stress and treatment with lipopolysaccharide or proinflammatory cytokines such as interleukin-1 and tumor necrosis factor. The cytokine biosynthesis inhibitors of Formula (I) have been determined to be potent and selective inhibitors of CSBP/p38/RK kinase activity. Patent Application USSN 08/123175 Lee et al., filed September 1993, USSN; Lee et al., PCT 94/10529 filed 16 September 1994 and Lee et al., *Nature* 300, n(72), 739-746 (Dec. 1994) whose disclosures are incorporated by reference herein in its entirety describes the above noted method for screening drugs to identify compounds which interact with and bind to the cytokine specific binding protein (hereinafter CSBP).

In order to use a compound of Formula (I) or a pharmaceutically acceptable salt thereof in therapy, it will normally be Formulated into a pharmaceutical composition in accordance with standard pharmaceutical practice. This invention, therefore, also relates to a pharmaceutical composition comprising an effective, non-toxic amount of a compound of Formula (I) and a pharmaceutically acceptable carrier or diluent.

Compounds of Formula (I), pharmaceutically acceptable salts thereof and pharmaceutical compositions incorporating such may conveniently be administered by any of the routes conventionally used for drug administration, for instance,

orally, topically, parenterally or by inhalation. The compounds of Formula (I) may be administered in conventional dosage forms prepared by combining a compound of Formula (I) with standard pharmaceutical carriers according to conventional procedures. The compounds of Formula (I) may also be administered in conventional dosages in combination with a known, second therapeutically active compound. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation. It will be appreciated that the form and character of the pharmaceutically acceptable character or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the Formulation and not deleterious to the recipient thereof.

The pharmaceutical carrier employed may be, for example, either a solid or liquid. Exemplary of solid carriers are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid carriers are syrup, peanut oil, olive oil, water and the like. Similarly, the carrier or diluent may include time delay material well known to the art, such as glyceryl mono-stearate or glyceryl distearate alone or with a wax.

A wide variety of pharmaceutical forms can be employed. Thus, if a solid carrier is used, the preparation can be tableted, placed in a hard gelatin capsule in powder or pellet form or in the form of a troche or lozenge. The amount of solid carrier will vary widely but preferably will be from about 25mg. to about 1g. When a liquid carrier is used, the preparation will be in the form of a syrup, emulsion, soft gelatin capsule, sterile injectable liquid such as an ampule or nonaqueous liquid suspension. It is recognized that compounds of Formula (I) can be administered by inhalation, orally, bucally, etc. and as such the previously noted patents which are incorporated by reference herein should be conferred.

It is expected that compounds of formula (I) may be administered parenterally, that is by intravenous, intramuscular, subcutaneous intranasal, intrarectal, intravaginal or intraperitoneal administration. The subcutaneous and intramuscular forms of the parenteral form administration are generally preferred.

Appropriate dosage forms for such administration may be prepared by conventional techniques. An oral administration is the most preferred route of treatment.

As used throughout the description of the methods for inhibiting myelopoiesis, the term "effective amount" refers to that amount of the compound of formula (I) which evokes a reversible inhibition of proliferating myelopoietic cells. In general, in order to exert an inhibitory effect, the compounds of the invention may be administered to human or other mammalian patients by injection in the dose range of about 0.5 mg to about 1000 mg. As one example, a desirable dosage range is from about 5 to about 1000 mg. The daily oral dosage regimen will be from about 0.1 to about 100 mg/kg of total body weight, preferably from about 0.2 to 50 mg/kg. If administered by infusion or similar means. the daily parenteral dosage regimen will be from about 0.1 to about 100 mg/kg of total body weight, preferably from about 0.2 to about 50 mg/kg. In principle, it is desirable to produce a concentration of the compound of about 10^{-9} M to about 10^{-5} M in the extracellular fluid of the patient, preferably 10^{-7} M.

For adaptation of the method for *ex vivo* or *in vitro* use of these compounds, e.g., for administration to bone marrow outside of the body of the patient donor, the effective amounts or dosages may be adjusted based on the amount of tissue being treated. Preferably, an effective *in vitro* concentration is in the range of about 10^{-9} M to 10^{-5} M. One of skill in the art may readily determine other appropriate dosages, depending on the mode of administration, and the level of aggressiveness of therapy required in the specific circumstance.

Another aspect of the present invention is a method for identifying or screening for compounds which inhibit CFU-C formation of myeloid progenitors, which method comprises the steps of:

(a) bringing together a test sample containing one or more test compounds and hematopoietic growth factors with a preparation comprising a receptor on myeloid progenitor cells capable of detectably and reversibly interacting with a compound of Formula (I) as defined herein, in soft agar;

(b) incubating said test sample and said preparation under conditions which would permit the detection of inhibition of CFU-C colony growth; and

(c) determining the degree of inhibition caused by said test sample by measuring MTT conversion by extracting said preparation and measuring optical density at 570 nm with a reference filter of 750 nm on an ELISA reader.

This method further comprises a step of comparing the degree of inhibition caused by said test sample with that caused by said compound. Preferably the receptor preparation comprises isolated myeloid progenitor cells. This method allows, preferably, for the preparation to be from bone marrow cells in soft agar.

BIOLOGICAL EXAMPLES SECTION

The following examples illustrate the preparation and uses of myeloprotectant compounds of this invention. These examples are illustrative only, and do not limit the scope of the present invention.

A. Micro-Inhibition Assay

One screening and identification method useful in the present invention may employ the step of screening test samples which detectably bind to a receptor for *in vitro* or *in vivo* inhibition of CFU-C colony formation in either a conventional 7 day CFU-GM assay (CFU-C Assay), well known to those skilled in the art, or in the novel, micro-screening assay as described herein. The micro-inhibition assay is a modification of the conventional assay and provides for an efficient and rapid screening and identification of inhibitors. The presence or amount of inhibition of CFU-C or CFU-GM colony formation can then be measured in order to identify those test samples which act as agonists. For example, in one such embodiment, an assay for the screening or identification of other myeloprotectant compounds capable of reversible inhibition includes the following steps. First, to measure the occurrence of, and degree of, inhibition, a test sample containing one or more test compounds and selected hematopoietic growth factors is contacted with bone marrow cells in soft agar (a semi-solid matrix) in a 96 well tissue culture plate. The

hematopoietic growth factor may be any of the known factors. Desirable factors specifically include M-CSF, IL-1, IL-3, stem cell factor (SCF), and/or a combination thereof.

The plate is then incubated under conditions which would permit the detection of inhibition of CFU-C CFU-C or CFU-GM colony growth. Such conditions include incubation at about 37°C humidified atmosphere of about 7% CO_2 for about 4 to 7 days. The degree of inhibition caused by the test sample is detected by measuring the conversion of the mitochondrial metabolism of (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide), also known as MTT, to an insoluble formazan crystal which can be dissolved in sodium dodecyl sulfate (SDS). Optical density is measured at 570 nm with a reference filter of 750 nm on an ELISA reader. The results of the assay with the peptide are compared to those of a control, i.e., bone marrow cells with growth factors and no peptide, as well as marrow cells only. Increased OD means increased MTT conversion indicative of increased cell metabolism or growth.

B. Micro-Reversibility Assay

An alternative assay method, called a pre-CFU-liquid culture (PCLC) assay, enables the determination of whether the test compound demonstrates reversibility of inhibition. In the PCLC assay or the novel micro reversibility assay as described below, the test sample is first contacted with bone marrow cells in a liquid medium with or without a test compound, in this instance a compound of formula (I), in a 96 well tissue culture plate for 1 to 4 days. Marrow cells are washed with medium three times and recultured in either a conventional CFU-C assay or the above noted Micro-Inhibition assay. The degree of inhibition caused by the test sample is again detected by measuring MTT conversion, as an index of CFU-C proliferation, and measuring optical density at 570 nm with a reference filter of 750 nm on an ELISA reader, using the same controls, as described above.

The assay methods may be further modified by preparing two or more additional test samples from the original test sample or samples that are determined

to inhibit CFU-C colony formation activity. These additional test samples contain a lesser number of test compounds than the original test sample from which they were prepared. The steps of the assays may then be repeated as many times as desired or until the test compound or compounds which bind to the receptor preparation have been identified.

Example 1 - Myeloprotectant Compound Demonstrates Bone Marrow CFU-C Inhibitory Activity *in vitro* in a Micro-Inhibition Assay

Dilutions of compound stock solution (DMSO) are made in phosphate buffered saline (PBS) with 0.01% bovine serum albumin. Different concentrations (5 uM, 0.5 uM, 50 nM, 5 nM) of Compound were added to wells in a maximum volume of 25 microliters. Non adherent bone marrow cells (4 x 10^4 cells/ 200 ul) were added in agar with 250 Units of M-CSF. These plates were grown in 37°C incubators in a humidified atmosphere of 7% CO_2 for 5 days.

All Micro-Inhibition plates were incubated with MTT for several hours and solubilized with SDS overnight. Plates were read at 570 nm with a reference of 750 nm. All values compared to wells containing media / agar/ and cells with no M-CSF growth factor.

The results of this assay demonstrate that compounds of Formula (I) inhibit murine bone marrow CFU-C growth. Bovine serum albumin (0.01%) represents background M-CSF stimulated CFU-C growth detected in this assay format. TGFβ-1 (25 ng/ml) was included as a positive control for CFU-C growth inhibition.

Compounds of Formula (I) which inhibited murine bone marrow CHU-C growth are:

1-(3'-N-Morpholino-1'-propyl)-4-(4'-fluorophenyl)-5-(4'-pyridyl)imidazole

5-(2-Amino-4-pyrimidinyl)-4-(4-fluorophenyl)-1-(1-methyl-4-piperidinyl)imidazole

5-(2-Amino-4-pyrimidinyl)-4-(4-fluorophenyl)-1-(2,2,6,6-tetramethyl-4-piperidinyl)imidazole

5-[4-(2-N-Methylamino)pyrimidinyl]-4-(4-fluorophenyl)-1-(4-N-methylpiperidine)imidazole

5-(2-Amino-4-pyrimidinyl)-4-(4-fluorophenyl)-1-(trans-4-hydroxycyclohexyl)imidazole

5-(2-Methylamino-4-pyrimidinyl)-4-(4-fluorophenyl)-1-(4-piperidinyl)imidazole

4-(4-Fluorophenyl)-5-(4-pyridyl)-1-(4-piperidinyl)imidazole

5-(2-Phenylamino-4-pyrimidinyl)-4-(4-fluorophenyl)-1-(4-piperidinyl)imidazole

Trans-1-(4-hydroxycyclohexyl)-4-(4-fluorophenyl)-5-[(2-methoxy)pyrimidin-4-yl]imidazole

5-(2-Phenoxy-4-pyrimidinyl)-4-(4-fluorophenyl)-1-(4-piperidinyl)imidazole

5-[4-(2-Hydroxy)pyrimidinyl]-4-(4-fluorophenyl)-1-(4-ketocyclohexyl)imidazole

1-(4-piperidinyl)-4-(4-fluorophenyl)-5-(2-phenoxy-4-pyridinyl)imidazole

1-(4-Piperidinyl)-4-(4-fluorophenyl)-5-(2-isopropoxy-4-pyrimidinyl) imidazole

1-(4-Piperidinyl)-4-(4-fluorophenyl)-5-(2-methoxy-4-pyrimidinyl)imidazole

1-(4-Piperidinyl)-[2-3H]-4-(4-fluorophenyl)-5- (2-methoxy-4-pyrimidinyl) imidazole

Trans-5-[4-(2-Phenoxy)pyrimidinyl]-4-(4-fluorophenyl)-1-(4-hydroxycyclohexyl)imidazole

1-(4-Piperidinyl)-4-(4-fluorophenyl)-5-(2-anilino-4-pyridinyl)imidazole

1-(Piperidin-4-yl)-4-(4-fluorophenyl)-5-[2-(4-methoxyphenoxy)pyrimidin-4-yl]imidazole

1-(Piperidin-4-yl)-4-(4-fluorophenyl)-5-[2-(4-fluorophenoxy)pyrimidin-4-yl]imidazole

1-(Piperidin-4-yl)-4-(4-fluorophenyl)-5-[2-(4-aminocarbonylphenoxy)pyrimidin-4-yl]imidazole

1-(Piperidin-4-yl)-4-(4-fluorophenyl)-5-[2-(4-ethylphenoxy)pyrimidin-4-yl]imidazole

1-(Piperidin-4-yl)-4-(4-fluorophenyl)-5-[2-(4-benzyloxyphenoxy)pyrimidin-4-yl]imidazole

1-(Piperidin-4-yl)-4-(4-fluorophenyl)-5-[2-(4-cyanophenoxy)pyrimidin-4-yl]imidazole

1-(Piperidin-4-yl)-4-(4-fluorophenyl)-5-[2-(4-hydroxyphenoxy)pyrimidin-4-yl]imidazole

Example 2 - Micro-Reversibility Assay - *In Vitro* Reversibility of CFU-C Inhibitory Activity

The Micro-Reversibility assay of this invention is performed as follows. 100,000 non-adherent murine bone marrow cells/ml were incubated for 24 hours with compound (5 uM, 0.5 uM, 50 nM, 5 nM) and a mixture of CSFs (mixture of IL-1, IL-3, SCF, M-CSF) in McCoys 5a liquid culture medium with 15% FBS. After 24 hours the cells were washed and resuspended in media / agar / and M-CSF and assayed according to the Micro-Inhibition assay of Example 1, above.

The results of this assay demonstrate that incubation of murine bone marrow cells with BSA buffer and CSF for 24 hours in liquid medium prior to washing and replating in a Micro-Inhibition assay results in measurable CFU-C growth as measured by MTT conversion. However, cells cultured for 24 hours in CSF and compound in liquid media, followed by washing and replating resulted in slightly greater CFU-C growth as measured by MTT conversion. There was no difference in the number of cells recovered from both sets of cultures. Marrow cells cultured with TGFb did not grow as well as BSA buffer of compound treated cells indicating an incomplete reversal of initial inhibitory effects of this concentration of TGFβ.

These data indicate the inhibition of CFU-C formation by compound is completely reversible at these doses and that the inhibition of CFU-C growth does not represent cytotoxicity. In the two graphs shown below as Figure 21, Compound 1 is 4-(4-Fluorophenyl)-5-(2-amino-4-pyridiminyl)-1-(1-methyl-4-piperidinyl)imidazole, and Compound 2 is 4-(4-Fluorophenyl)-5-(2-phenylamino-4-pyridiminyl)-1-(4-piperidinyl)imidazole.

Example 3 - *In Vivo* Inhibitory Activity of a Myeloprotectant Compound on CFU-C Formation

The present invention designed studies to prove whether the compounds of Formula (I) were reversible inhibitors of CFU-GM and hence myeloprotective in vivo in a high dose 5-FU model as described herein. Stock solutions are diluted in PBS prior to injection. The test compound concentration is adjusted so that a 0.2 ml injection delivers the appropriate dose of compound.

To assess the *in vivo* inhibition of CFU-C formation, Female BDF1 mice were injected IP, (or the dosage may be given orally) with 10 mg/kg 1 hour prior to and 3 hours after 175 mg/kg 5-FU. Mice were sacrificed on days 3, 4, and 6 post 5-FU injection. Bone marrow cellularity and CFU-GM analysis was performed on each day. Data shown in Figure 1 demonstrate that 5-FU severely depleted the marrow compartment of CFU-GM evident on days 3 and 4. By day 6 recovery has initiated, however CFU-GM content is less than 5% of normal. Mice injected with either 4-(4-Fluorophenyl)-5-(2-phenylamino-4-pyridiminyl)-1-(4-piperidinyl)imidazole as Compound 2 or 4-(4-Fluorophenyl)-5-(2-N-methylamino-4-pyridiminyl)-1-(4-N-methyl-piperidinyl)imidazoleas Compound 3. Both Compound 2 and Compound 3 had significantly greater numbers of CFU-GM in the femur at each day tested. On day 4 the CFU-GM content of the marrow was approximately 50% of normal values. This experiment was repeated twice more at day 3 resulting in the same conclusions. Administration of either Compound 2 or at these doses protected a proportion of CFU-GM from 5-FU toxicity. Similar to Compounds 2 and 3 and oral dosage of Compound 4, 4-(4-Fluorophenyl)-5-(2-N-methylamino-4-pyridiminyl)-1-(4-piperidinyl)imidazole at levels of 50 mg/kg resulted in the same protection.

Example 4 - A Myeloprotectant Compound Effects Stem Cells Post 5-Fluorouracil Myelosuppression

One of the utilities of a myeloprotectant agent is for prevention of neutrophil nadir or a more rapid recovery of neutrophils post chemotherapeutic insult.

Administration of either Compound 2 or 3 resulted in significant increases in mature blood cell elements and bone marrow cellularity on day 9 post 5-FU. While the pattern / kinetics of activity for these two compounds were not identical, the specific cell lineages were more effected by one compound and not the other, however both compounds were effective as myeloprotectants. Shown in Figure 2, below Compound 3, in a dose responsive manner increased total white blood cell (WBC) count over 5-FU. In Figure 3, Compound 2 in a dose responsive manner, increased neutrophil counts to levels equivalent to normal non-5-FU treated mice. Compound 3, also significantly increased PMN in a dose related manner.

In Figure 4, both compounds increased platelet counts in a dose responsive manner. Platelet numbers in the 5-FU model, decrease by 60% than rebound to values higher than normal on days 10 - 15. In this experiment, the 5-FU and normal platelets counts are equivalent as the 5-FU counts are beginning this rebound phase of platelet production. Both compounds resulted in higher PLT counts at day 9 indicating that PLT recovery was quicker in the CSAID™ compound treated mice. In figures 5, 6 and 7 Compounds 2 and 3 significantly increased lymphocyte, monocyte, and RBC counts in a dose responsive manner. These data taken together indicate that multiple hematopoietic cell lineage's are protected by administration of compounds of Formula (I) in this murine 5-FU model. This pattern of mature blood cell recovery is unprecedented. No known agent / drug (i.e. G-CSF) increases every cell lineage in chemotherapy models of myelosuppression. In addition to mature blood cell elements, bone marrow cellularity was also increased in a dose responsive manner (Figure 8) indicating myeloprotection and enhanced cell recovery within the marrow. This study (Figure 8) was repeated with Compounds 2 and 3 administered orally. Figures 9 and 10, show only the PMN and marrow cellularity data. Both compounds appear to be more effective post oral administration than IP.

In order to determine whether a compound could increase PMN could increase mature blood cell counts the following experiment was done. In a preliminary experiment compounds of Formula (I), Compounds 2 and 3 increased PMN counts on day 9 post 5-FU. Therefore, day 9 post 5-FU was chosen as an

indicator time because mature blood cell counts are still significantly suppressed in 5-FU treated mice at this time. In addition, from previous experiments it was known that G-CSF administered every day post 5-FU begins to increase PMN counts starting around day 9 - 10. Based on these results a dose response analysis of Compounds 2 and 3 were performed utilizing the Day 9 5-FU myelosuppression model shown herein. Similar to Example 3 above, mice were injected IP with compound 1 hour prior to and 3 hours after the cell cycle specific drug, 5-fluorouracil (5-FU 175mg/kg), which occurred at 0 hour. The timing of the compound injections in the figures 2 to 10 were made wherein the compound was injected IP 2 hours prior and 4 hours after the myelosuppresive dose was administered.

Example 5: Evaluation of Compounds of Formula (I) and G-CSF in a murine 5-FU myelosuppression model

As shown above compounds of Formula (I) are effective reversible inhibitors of CFU-GM growth *in vitro* and *in vivo*. Examples 1 to 4, above demonstrate that compounds of Formula (I) in experiments in which a high dose of 5-FU (175 mg/kg) is are myeloprotectants for normal hematopoietic stem cells. The following experiments demonstrate the myeloprotective effects of compounds of Formula (I), in particular Compounds 2 and 3, in a kinetic model of 5-FU induced myelosuppression.

G-CSF (50 ug/kg administered daily 48 hours post 5-FU) is included as a positive control for enhanced PMN recovery in these studies. It should be noted that G-CSF is administered 1.5 hours before each bleed point. G-CSF administration under these conditions reflects PMN recovery plus additional PMN mobilization from vascular and possibly tissue sites.

Administration of 5-FU resulted in significant neutropenia lasting 8 days. All mature cell elements were depleted by 5-FU, however the extent and duration vary with each cell lineage. Administration of either Compound 2 or 3 (IP, 40 mg/kg; 2 hours pre and 4 hours post 5-FU) resulted in enhanced mature blood cell

recovery across multiple lineage's. The most dramatic effects were on PMN, monocytes, and platelet recoveries. Data shown in Figures 11 through 16.

Administration of either Compound 2 or 3, 2 hours before and 4 hours after high dose 5-FU significantly enhance overall mature blood cell recovery. The data from the above Figures can be summarized as the following table of hematology values. The lower the value the faster the cell lineage has recovered. Compounds 2 or 3 were effective myeloprotectants with activity better or equal to that of therapeutic G-CSF.

TABLE 2. Summary Table of Key Hematology End Points in the Murine 5-FU Myeloprotection Model. Values extrapolated from the kinetic study graphs.

Parameter	5-FU Alone	G-CSF	Cmpd. II	Cmpd. III
PMN Days under 300	5	5	3	3
PMN Days till normal	13	9.75	9.5	11
MONO Days under 50	7	5	3	2
MONO Days till normal	12.5	11.5	9.75	11.5
Platelet Days till normal	8	8	6.75	7.25

Example 6: Evaluation of Compound of Formula (I) alone or in combination with therapeutic G-CSF in a high dose 5-FU model

Compounds of Formula (I) have shown efficacy as myeloprotectants in a murine high dose 5-FU model noted above. In this experiment, Compound 3 (40 mg/kg) was administered IP to mice 2 hours before and 4 hours after a single

injection of 175 mg/kg 5-FU G-CSF was administered daily starting 48 hours post 5-FU injection for 10 days.

As shown in earlier experiments, IP administration of Compound 3 is effective as a myeloprotectant, and the data demonstrates that a trend towards rapid recovery were seen in every cell lineage. G-CSF alone resulted in enhanced recovery of PMN and monocyte populations as well as total WBC's. The combination of a compound of Formula (I) and pre-treatment with G-CSF therapy resulted in dramatic increases in multiple cell lineage recovery rates. The most dramatic increase in recovery rate was the PMN population with evidence over several days of complete protection of neutropenia in a subpopulation of treated mice. These data are shown in Figures 17 to 20 herein.

Consequently another aspect of the present invention is the novel administration of a compound of Formula (I) along with G-CSF for the protection of multiple lineage cell lines and their enhanced recovery.

Overall mature blood cell recovery and is summarized as the following table of hematology values. The lower the value the faster the cell lineage has recovered. Compound 3 + therapeutic G-CSF [QDx10] were effective myeloprotectants with activity better or equal to that of G-CSF alone.

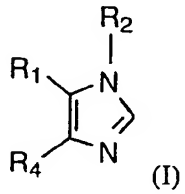
Parameter	5-FU Alone	G-CSF	Cmpd. III	Cmpd. III + G-CSF
PMN Days under 300	6	5	6	0
PMN Days till normal	13.5	10.25	13.5	3.5
MONO Days under 50	6	6	5	1
MONO Days till normal	13	11.5	12	10.75
Platelet Days till normal	8.5	8.5	8	8.25

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

The above description fully discloses the invention including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further elaboration, it is believed that one skilled in the are can, using the preceding description, utilize the present invention to its fullest extent. Therefore the Examples herein are to be construed as merely illustrative and not a limitation of the scope of the present invention in any way. The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.

What is claimed is:

1. A method for reversibly inhibiting myelopoiesis in mammalian tissue *in vivo* and *ex vivo* comprising administering to said tissue an amount effective to reversibly inhibit the formation of myelopoietic colonies during the time said tissue is exposed to myelosuppressive therapy of a compound of the formula:



(I)

wherein:

R_1 is 4-pyridyl, pyrimidinyl, 4-pyridazinyl, 1,2,4-triazin-5-yl, quinolyl, isoquinolinyl, quinazolin-4-yl, 1-imidazolyl or 1-benzimidazolyl ring, which ring is optionally substituted independently one to three times with Y, NHR_a, optionally substituted C_{1-4} alkyl, halogen, hydroxyl, optionally substituted C_{1-4} alkoxy, optionally substituted C_{1-4} alkylthio, C_{1-4} alkylsulfinyl, CH_2OR_{12}, amino, mono and di- C_{1-6} alkyl substituted amino, or $N(R_{10})C(O)R_b$;

Y is $O\text{-}R_a$;

R_4 is phenyl, naphth-1-yl or naphth-2-yl, or a heteroaryl, which is optionally substituted by one or two substituents, each of which is independently selected, and which, for a 4-phenyl, 4-naphth-1-yl, 5-naphth-2-yl or 6-naphth-2-yl substituent, is halogen, cyano, nitro, $C(Z)NR_7R_{17}$, $C(Z)OR_{16}$, $(CR_{10}R_{20})_vCOR_{12}$, SR_5, SOR_5, OR_{12}, halo-substituted-C_{1-4} alkyl, C_{1-4} alkyl, $ZC(Z)R_{12}$, $NR_{10}C(Z)R_{16}$, or $(CR_{10}R_{20})_vNR_{10}R_{20}$ and which, for other positions of substitution, is halogen, cyano, $C(Z)NR_{13}R_{14}$, $C(Z)OR_3$, $(CR_{10}R_{20})_{m''}COR_3$, $S(O)_mR_3$, OR_3, halo-substituted-C_{1-4} alkyl, C_{1-4} alkyl, $(CR_{10}R_{20})_{m''}NR_{10}C(Z)R_3$, $NR_{10}S(O)_{m'}R_8$, $NR_{10}S(O)_{m'}NR_7R_{17}$, $ZC(Z)R_3$ or $(CR_{10}R_{20})_{m''}NR_{13}R_{14}$;

v is 0, or an integer having a value of 1 or 2;

n is an integer having a value of 1 to 10;

n' is 0, or an integer having a value of 1 to 10;

m is 0, or the integer 1 or 2;

m' is an integer having a value of 1 or 2,

m" is 0, or an integer having a value of 1 to 5;

R_2 is hydrogen, $(CR_{10}R_{20})_{n'}OR_9$, heterocyclyl, heterocyclylC_{1-10} alkyl, C_{1-10}alkyl, halo-substituted C_{1-10} alkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, C_{3-7} cycloalkyl, C_{3-7}cycloalkylC_{1-10} alkyl, C_{5-7} cycloalkenyl C_{1-10}alkyl, aryl, arylC_{1-10} alkyl, heteroaryl, heteroarylC_{1-10}alkyl, $(CR_{10}R_{20})_nOR_{11}$, $(CR_{10}R_{20})_nS(O)_mR_{18}$, $(CR_{10}R_{20})_nNHS(O)_2R_{18}$, $(CR_{10}R_{20})_nNR_{13}R_{14}$, $(CR_{10}R_{20})_nNO_2$, $(CR_{10}R_{20})_nCN$, $(CR_{10}R_{20})_{n'}SO_2R_{18}$, $(CR_{10}R_{20})_nS(O)_{m'}NR_{13}R_{14}$, $(CR_{10}R_{20})_nC(Z)R_{11}$, $(CR_{10}R_{20})_nOC(Z)R_{11}$, $(CR_{10}R_{20})_nC(Z)OR_{11}$, $(CR_{10}R_{20})_nC(Z)NR_{13}R_{14}$, $(CR_{10}R_{20})_nC(Z)NR_{11}OR_9$, $(CR_{10}R_{20})_nNR_{10}C(Z)R_{11}$, $(CR_{10}R_{20})_nNR_{10}C(Z)NR_{13}R_{14}$, $(CR_{10}R_{20})_nN(OR_6)C(Z)NR_{13}R_{14}$, $(CR_{10}R_{20})_nN(OR_6)C(Z)R_{11}$, $(CR_{10}R_{20})_nC(=NOR_6)R_{11}$, $(CR_{10}R_{20})_nNR_{10}C(=NR_{19})NR_{13}R_{14}$, $(CR_{10}R_{20})_nOC(Z)NR_{13}R_{14}$, $(CR_{10}R_{20})_nNR_{10}C(Z)NR_{13}R_{14}$, $(CR_{10}R_{20})_nNR_{10}C(Z)OR_{10}$, 5-$(R_{18})$-1,2,4-oxadizaol-3-yl or 4-(R_{12})-5-$(R_{18}R_{19})$-4,5-dihydro-1,2,4-oxadiazol-3-yl;

wherein the aryl, arylalkyl, cycloalkyl, cycloalkylalkyl, heteroaryl, heteroaryl alkyl, heterocyclic and heterocyclic alkyl groups may be optionally substituted;

Z is oxygen or sulfur;

R_a is C_{1-6}alkyl, aryl, arylC_{1-6}alkyl, heterocyclic, heterocyclylC_{1-6} alkyl, heteroaryl, or heteroarylC_{1-6}alkyl, wherein each of these moieties may be optionally substituted;

R_b is hydrogen, C_{1-6} alkyl, C_{3-7} cycloalkyl, aryl, arylC_{1-4} alkyl, heteroaryl, heteroarylC_{1-4}alkyl, heterocyclyl, or heterocyclylC_{1-4} alkyl;

R_3 is heterocyclyl, heterocyclylC_{1-10} alkyl or R_8;

R_5 is hydrogen, C_{1-4} alkyl, C_{2-4} alkenyl, C_{2-4} alkynyl or NR_7R_{17}, excluding the moieties $-SR_5$ being $-SNR_7R_{17}$ and $-SOR_5$ being -SOH;

R_6 is hydrogen, a pharmaceutically acceptable cation, C_{1-10} alkyl, C_{3-7} cycloalkyl, aryl, arylC_{1-4} alkyl, heteroaryl, heteroarylC_{1-4} alkyl, heterocyclic, aroyl, or C_{1-10} alkanoyl;

R_7 and R_{17} is each independently selected from hydrogen or C_{1-4} alkyl or R_7 and R_{17} together with the nitrogen to which they are attached form a heterocyclic ring of 5 to 7 members which ring optionally contains an additional heteroatom selected from oxygen, sulfur or NR_{15};

R8 is C_{1-10} alkyl, halo-substituted C_{1-10} alkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, C_{3-7} cycloalkyl, C_{5-7} cycloalkenyl, aryl, arylC_{1-10} alkyl, heteroaryl, heteroarylC_{1-10} alkyl, $(CR_{10}R_{20})_nOR_{11}$, $(CR_{10}R_{20})_nS(O)_mR_{18}$, $(CR_{10}R_{20})_nNHS(O)_2R_{18}$, $(CR_{10}R_{20})_nNR_{13}R_{14}$; wherein the aryl, arylalkyl, heteroaryl, heteroaryl alkyl may be optionally substituted;

R9 is hydrogen, $C(Z)R_{11}$ or optionally substituted C_{1-10} alkyl, $S(O)_2R_{18}$, optionally substituted aryl or optionally substituted aryl-C_{1-4} alkyl;

R_{10} and R_{20} is each independently selected from hydrogen or C_{1-4} alkyl;

R_{11} is hydrogen, C_{1-10} alkyl, C_{3-7} cycloalkyl, heterocyclyl, heterocyclyl C_{1-10}alkyl, aryl, arylC_{1-10} alkyl, heteroaryl or heteroarylC_{1-10} alkyl;

R_{12} is hydrogen or R_{16};

R_{13} and R_{14} is each independently selected from hydrogen or optionally substituted C_{1-4} alkyl, optionally substituted aryl or optionally substituted aryl-C_{1-4} alkyl, or together with the nitrogen to which they are attached form a heterocyclic ring of 5 to 7 members which ring optionally contains an additional heteroatom selected from oxygen, sulfur or NR9;

R_{15} is R_{10} or C(Z)-C_{1-4} alkyl;

R_{16} is C_{1-4} alkyl, halo-substituted-C_{1-4} alkyl, or C_{3-7} cycloalkyl;

R_{18} is C_{1-10} alkyl, C_{3-7} cycloalkyl, heterocyclyl, aryl, arylalkyl, heterocyclyl, heterocyclyl-C_{1-10}alkyl, heteroaryl or heteroarylalkyl;

R_{19} is hydrogen, cyano, C_{1-4} alkyl, C_{3-7} cycloalkyl or aryl;

or a pharmaceutically acceptable salt thereof.

2. The method according to claim 1 wherein said myelopoietic colonies are CFU-C colony forming cells.

3. The method according to claim 2 wherein said CFU-C colonies are CFU-GM colony forming cells.

4. The method according to claim 1 wherein reversible inhibition occurs *in vivo*.

5. The method according to claim 1 wherein the compound is administered to mammalian tissue *ex vivo* and wherein said reversible inhibition occurs *ex vivo*.

6. The method according to claim 1 for reversibly inhibiting myelopoiesis in mammalian tissue *in vivo* and *ex vivo* comprising administering to said tissue an effective amount of a compound of Formula (I).

7. The method according to anyone one of Claims 1 to 6 wherein the compound, or a pharmaceutically acceptable salt thereof is:
4-(4-Fluorophenyl)-5-(2-amino-4-pyridiminyl)-1-(1-methyl-4-piperidinyl)imidazole,
4-(4-Fluorophenyl)-5-(2-phenylamino-4-pyridiminyl)-1-(4-piperidinyl)imidazole;
4-(4-Fluorophenyl)-5-(2-N-methylamino-4-pyridiminyl)-1-(4-N-methyl-piperidinyl)imidazole; or
4-(4-Fluorophenyl)-5-(2-N-methylamino-4-pyridiminyl)-1-(4-piperidinyl)imidazole.

8. The method according to Claim 1 wherein the compound is administered in an oral dosage form.

9. The method according to Claim 1 wherein the compound is administered in a parenteral dosage form.

10. A method for identifying or screening for compounds which inhibit CFU-C formation of myeloid progenitors which comprises the steps of:

(a) bringing together a test sample containing one or more test compounds and hematopoietic growth factors with a preparation comprising a receptor on myeloid progenitor cells capable of detectably and reversibly interacting with a compound of Formula (I), according to Claim 1, in soft agar;

(b) incubating said test sample and said preparation under conditions which would permit the detection of inhibition of CFU-C colony growth; and

(c) determining the degree of inhibition caused by said test sample by measuring MTT conversion by extracting said preparation and measuring optical density at 570 nm with a reference filter of 750 nm on an ELISA reader.

11. The method according to claim 10 comprising a further step of comparing the degree of inhibition caused by said test sample with that caused by a compound of Formula (I) as defined in Claim 1.

12. The method according to claim 10 wherein said receptor preparation comprises isolated myeloid progenitor cells.

13. The method according to claim 10 wherein said preparation is bone marrow cells in soft agar.

14. The method according to claim 10 wherein said test sample comprises one or more known compounds.

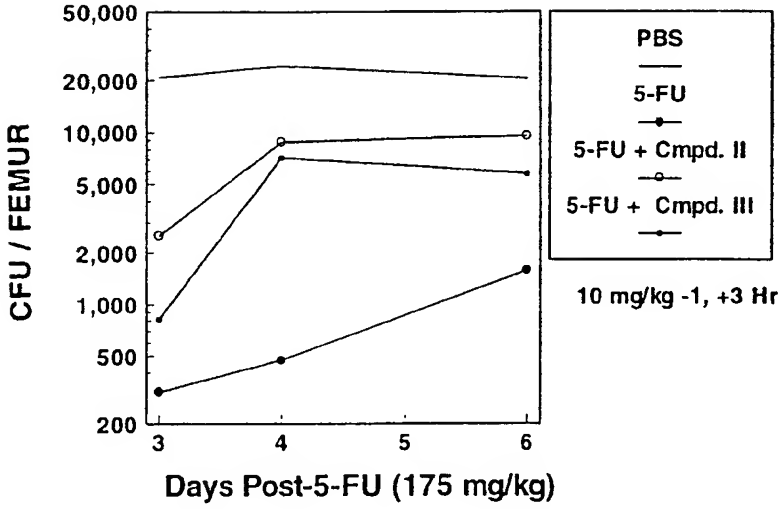

50,000
20,000
10,000
5,000
2,000
1,000
500
200
CFU / FEMUR
3
4
5
6
Days Post-5-FU (175 mg/kg)
PBS
5-FU
5-FU + Cmpd. II
5-FU + Cmpd. III
10 mg/kg -1, +3 Hr

Figure 1

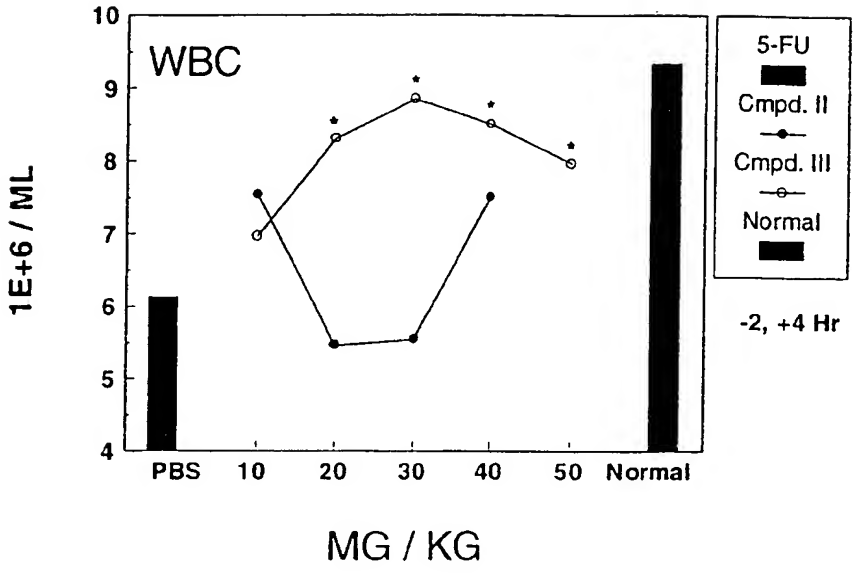

WBC
1E+6 / ML
10
9
8
7
6
5
4
PBS
10
20
30
40
50
Normal
MG / KG
5-FU
Cmpd. II
Cmpd. III
Normal
-2, +4 Hr

Figure 2

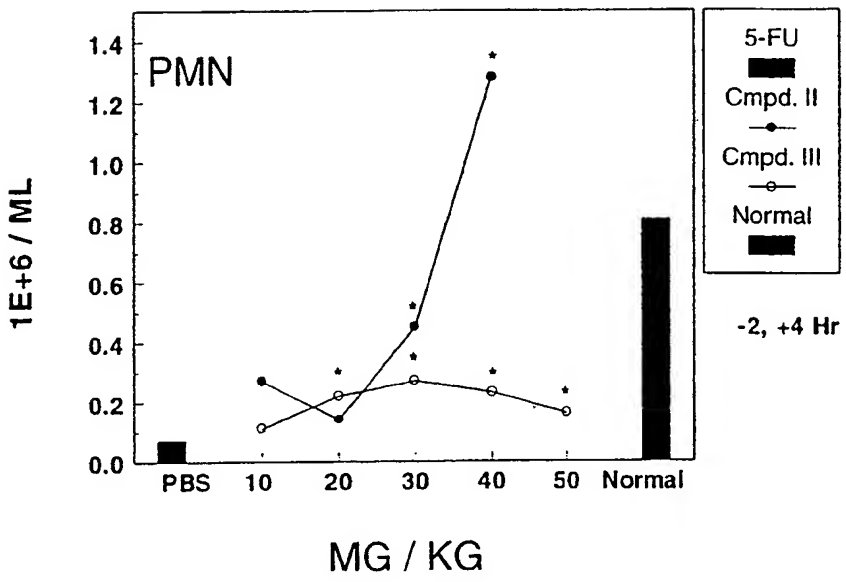

PMN
1E+6 / ML
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
PBS
10
20
30
40
50
Normal
MG / KG
5-FU
Cmpd. II
Cmpd. III
Normal
-2, +4 Hr

Figure 3

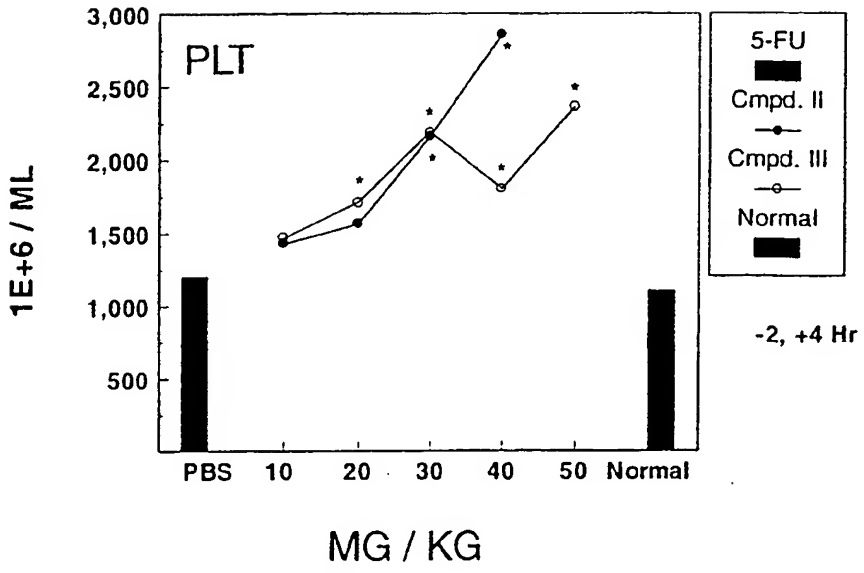

PLT
3,000
2,500
2,000
1,500
1,000
500
1E+6 / ML
PBS
10
20
30
40
50
Normal
MG / KG
5-FU
Cmpd. II
Cmpd. III
Normal
-2, +4 Hr

Figure 4

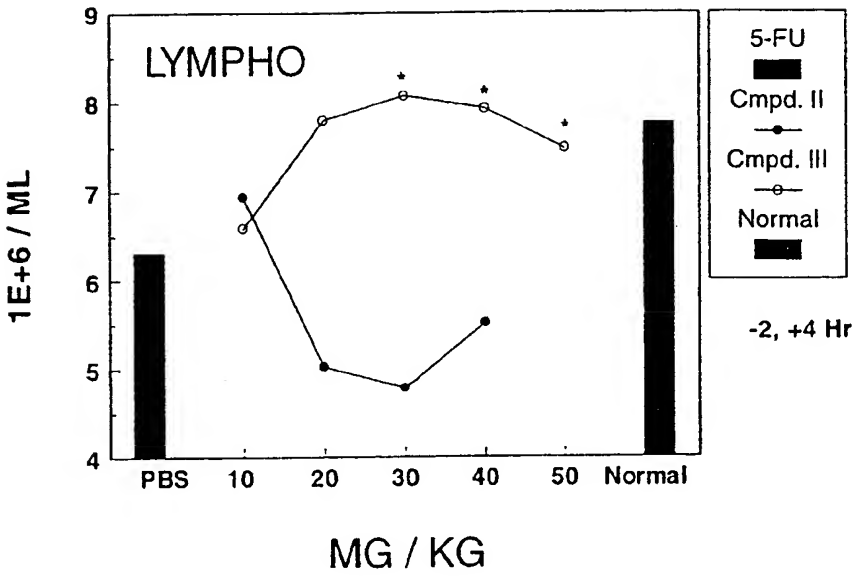

LYMPHO
1E+6 / ML
9
8
7
6
5
4
PBS
10
20
30
40
50
Normal
MG / KG
5-FU
Cmpd. II
Cmpd. III
Normal
-2, +4 Hr

Figure 5

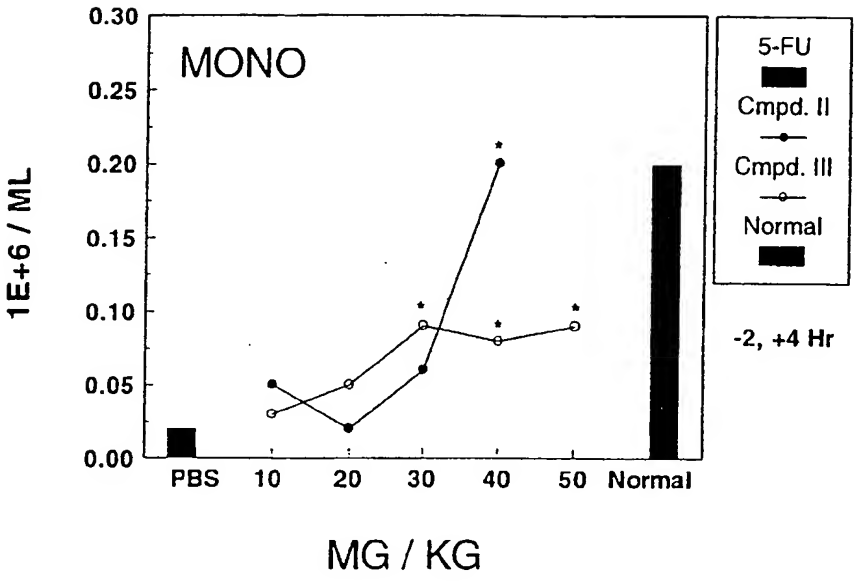

MONO
0.30
0.25
0.20
0.15
0.10
0.05
0.00
1E+6 / ML
PBS
10
20
30
40
50
Normal
MG / KG
5-FU
Cmpd. II
Cmpd. III
Normal
-2, +4 Hr

Figure 6

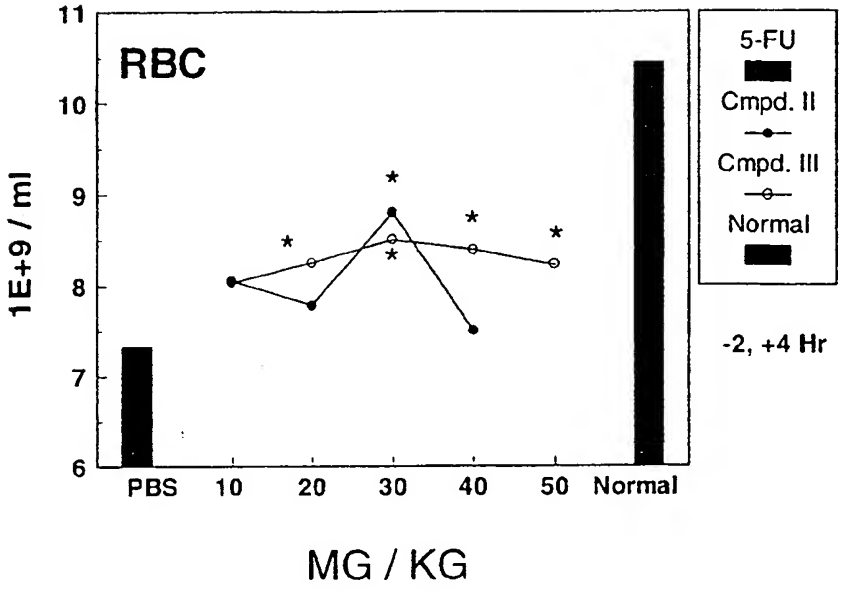

RBC
1E+9 / ml
11
10
9
8
7
6
PBS
10
20
30
40
50
Normal
MG / KG
5-FU
Cmpd. II
Cmpd. III
Normal
-2, +4 Hr

Figure 7

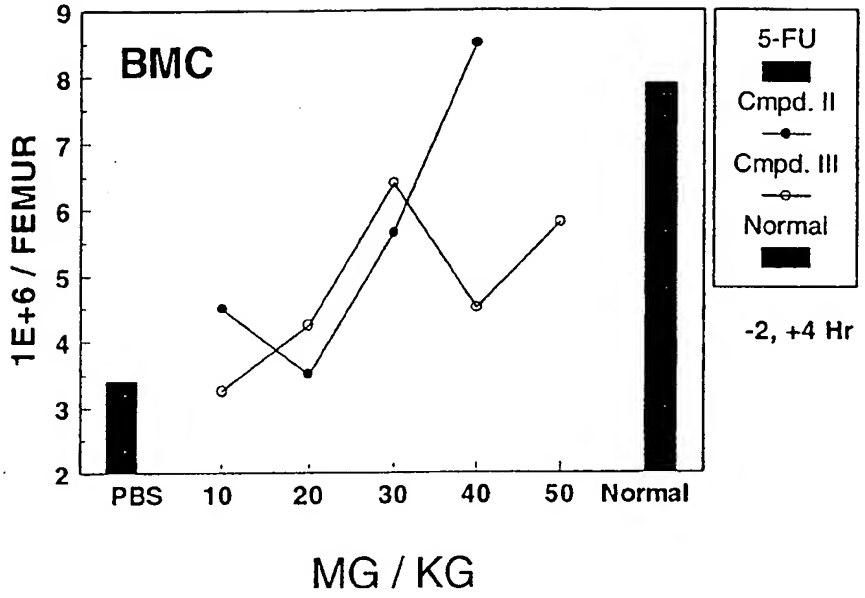

BMC
1E+6 / FEMUR
9
8
7
6
5
4
3
2
PBS
10
20
30
40
50
Normal
MG / KG
5-FU
Cmpd. II
Cmpd. III
Normal
-2, +4 Hr

Figure 8

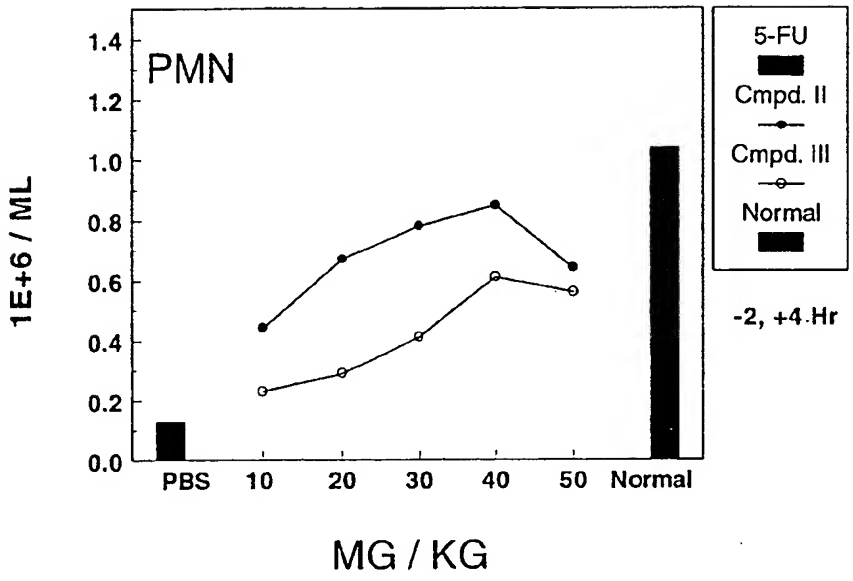

PMN
1E+6 / ML
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
PBS
10
20
30
40
50
Normal
MG / KG
5-FU
Cmpd. II
Cmpd. III
Normal
-2, +4 Hr

Figure 9

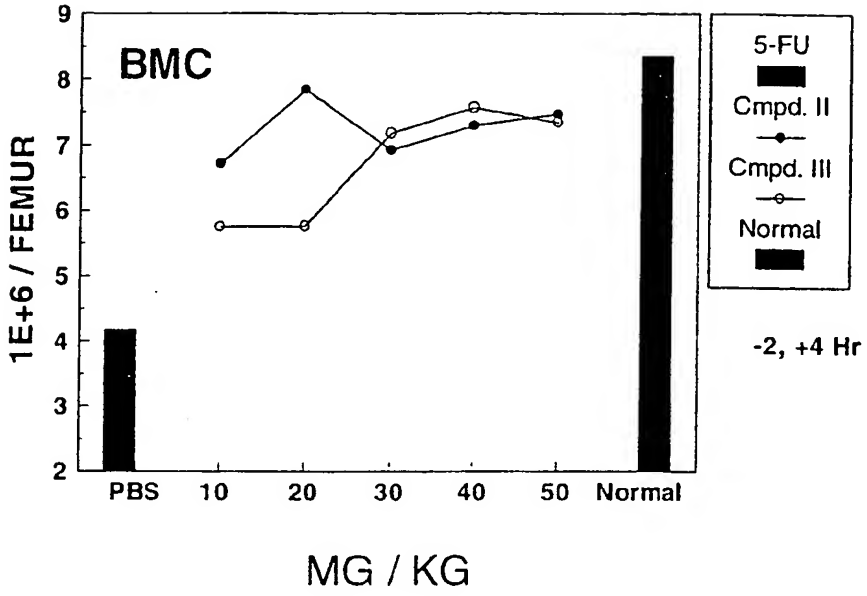

BMC
1E+6 / FEMUR
9
8
7
6
5
4
3
2
PBS
10
20
30
40
50
Normal
MG / KG
5-FU
Cmpd. II
Cmpd. III
Normal
-2, +4 Hr

Figure 10

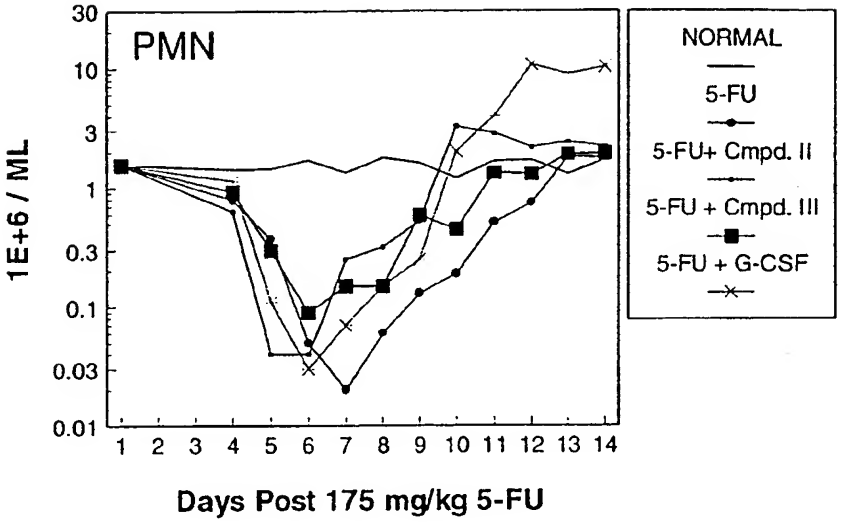

PMN
1E+6 / ML
30
10
3
1
0.3
0.1
0.03
0.01
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days Post 175 mg/kg 5-FU
NORMAL
5-FU
5-FU+ Cmpd. II
5-FU + Cmpd. III
5-FU + G-CSF

Figure 11

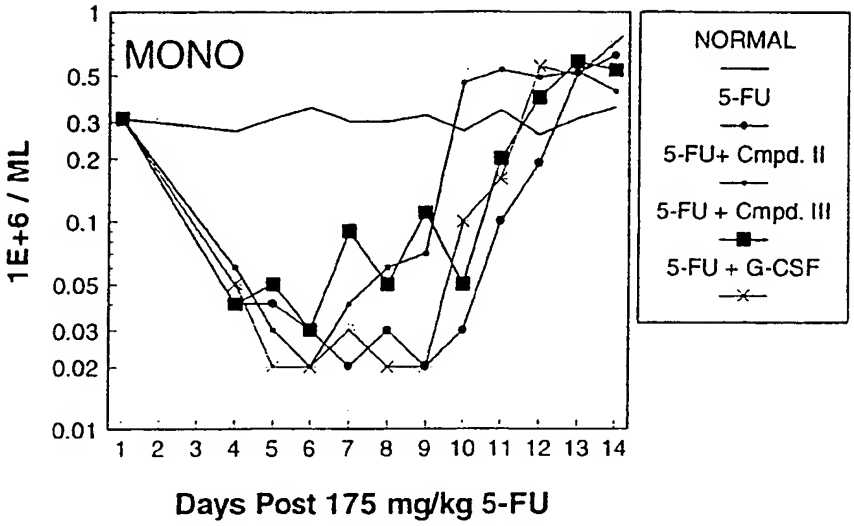

MONO
1E+6 / ML
1
0.5
0.3
0.2
0.1
0.05
0.03
0.02
0.01
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days Post 175 mg/kg 5-FU
NORMAL
5-FU
5-FU+ Cmpd. II
5-FU + Cmpd. III
5-FU + G-CSF

Figure 12

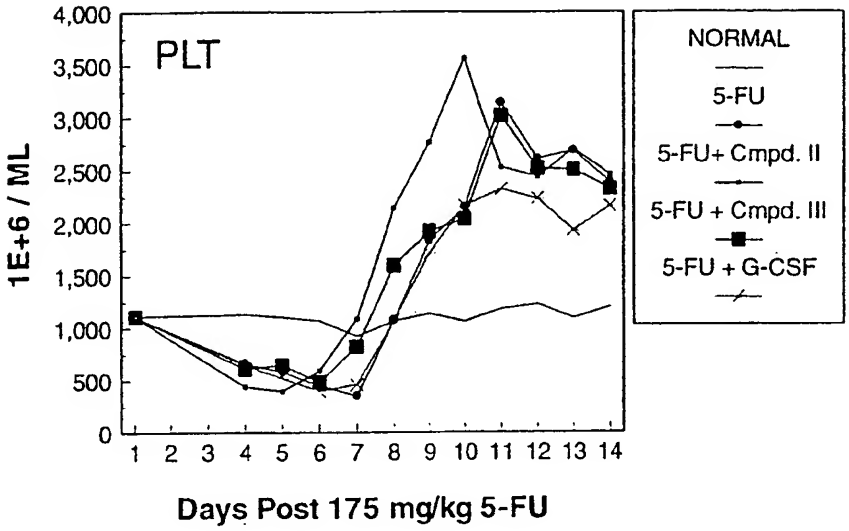

PLT
1E+6 / ML
4,000
3,500
3,000
2,500
2,000
1,500
1,000
500
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days Post 175 mg/kg 5-FU
NORMAL
5-FU
5-FU+ Cmpd. II
5-FU + Cmpd. III
5-FU + G-CSF

Figure 13

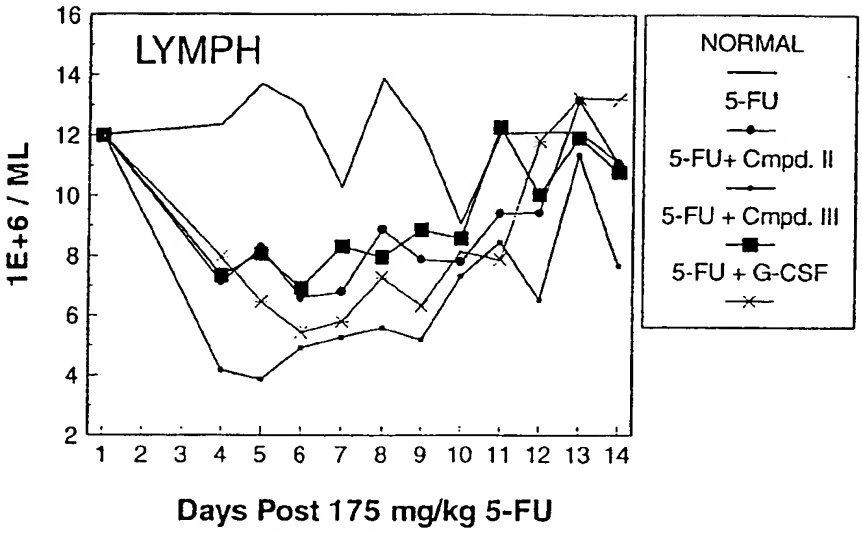

LYMPH
1E+6 / ML
16
14
12
10
8
6
4
2
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days Post 175 mg/kg 5-FU
NORMAL
5-FU
5-FU+ Cmpd. II
5-FU + Cmpd. III
5-FU + G-CSF

Figure 14

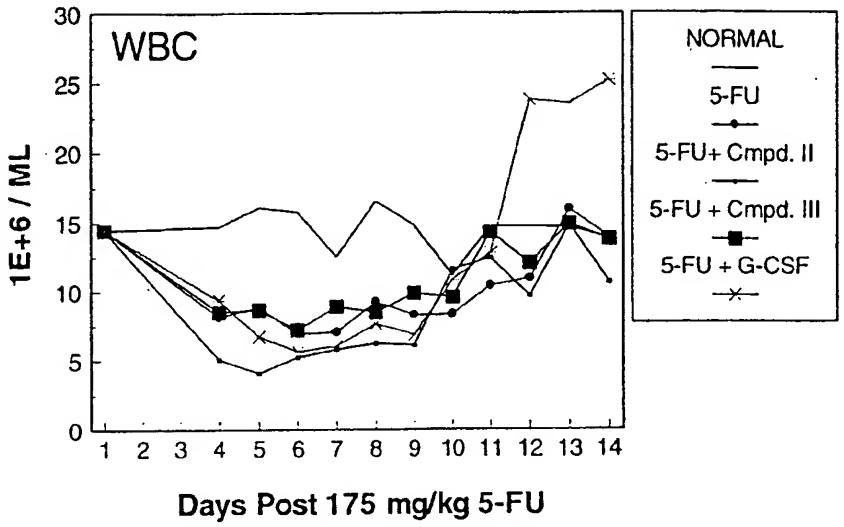

WBC
1E+6 / ML
30
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days Post 175 mg/kg 5-FU
NORMAL
5-FU
5-FU+ Cmpd. II
5-FU + Cmpd. III
5-FU + G-CSF

Figure 15

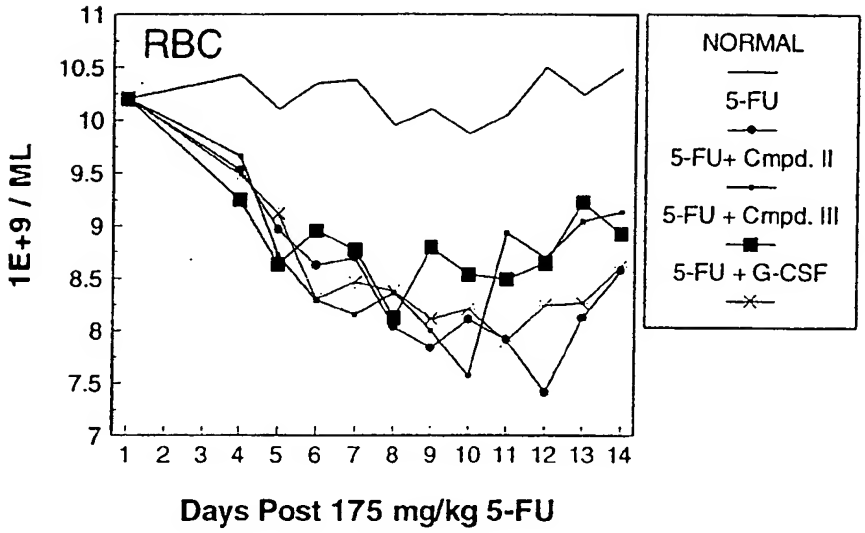

RBC
1E+9 / ML
11
10.5
10
9.5
9
8.5
8
7.5
7
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days Post 175 mg/kg 5-FU
NORMAL
5-FU
5-FU+ Cmpd. II
5-FU + Cmpd. III
5-FU + G-CSF

Figure 16

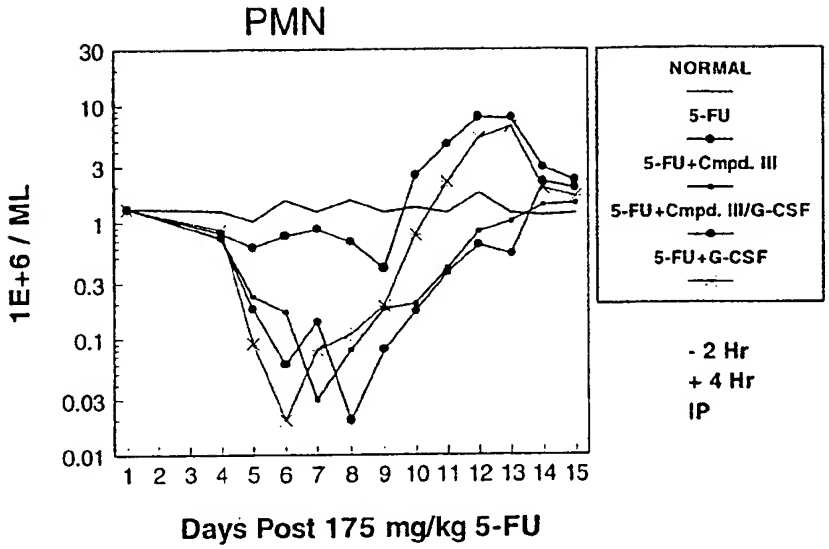

PMN
30
10
3
1
0.3
0.1
0.03
0.01
1E+6 / ML
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Days Post 175 mg/kg 5-FU
NORMAL
5-FU
5-FU+Cmpd. III
5-FU+Cmpd. III/G-CSF
5-FU+G-CSF
- 2 Hr
+ 4 Hr
IP

Figure 17

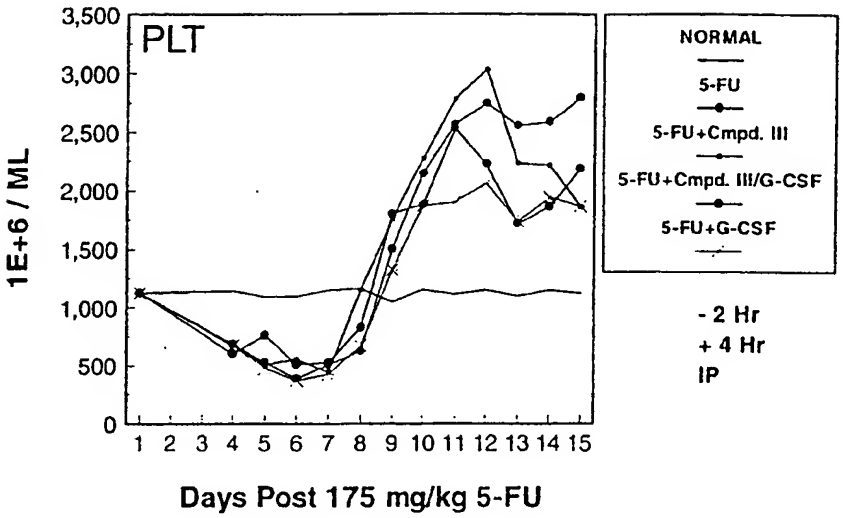

PLT
1E+6 / ML
3,500
3,000
2,500
2,000
1,500
1,000
500
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Days Post 175 mg/kg 5-FU
NORMAL
5-FU
5-FU+Cmpd. III
5-FU+Cmpd. III/G-CSF
5-FU+G-CSF
- 2 Hr
+ 4 Hr
IP

Figure 18

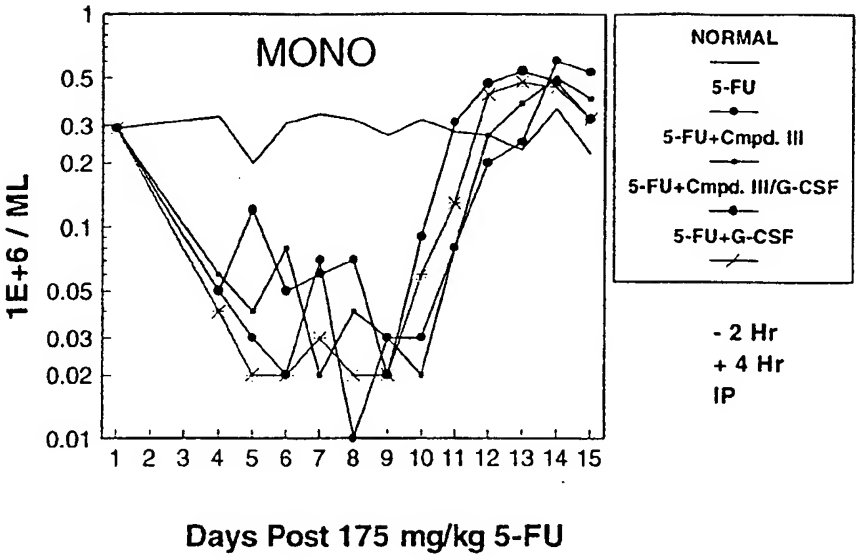

MONO
1E+6 / ML
1
0.5
0.3
0.2
0.1
0.05
0.03
0.02
0.01
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Days Post 175 mg/kg 5-FU
NORMAL
5-FU
5-FU+Cmpd. III
5-FU+Cmpd. III/G-CSF
5-FU+G-CSF
- 2 Hr
+ 4 Hr
IP

Figure 19

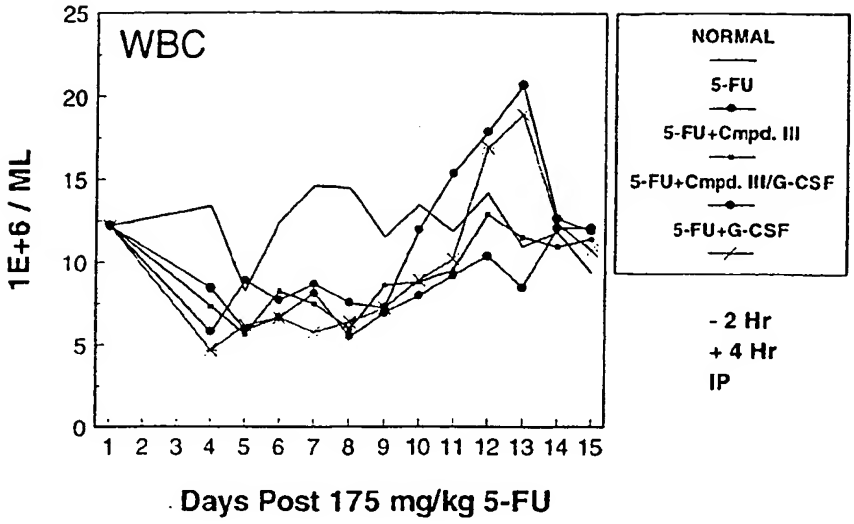

WBC
1E+6 / ML
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Days Post 175 mg/kg 5-FU
NORMAL
5-FU
5-FU+Cmpd. III
5-FU+Cmpd. III/G-CSF
5-FU+G-CSF
- 2 Hr
+ 4 Hr
IP

Figure 20

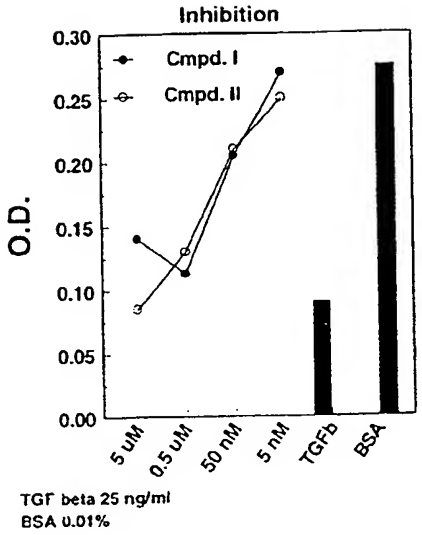

Inhibition
Cmpd. I
Cmpd. II
O.D.
0.30
0.25
0.20
0.15
0.10
0.05
0.00
5 uM
0.5 uM
50 nM
5 nM
TGFb
BSA
TGF beta 25 ng/ml
BSA 0.01%

Figure 21A

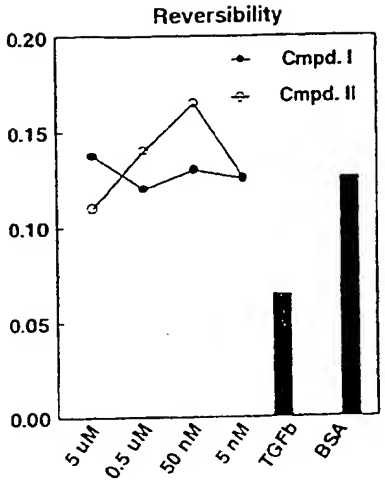

Reversibility
0.20
0.15
0.10
0.05
0.00
Cmpd. I
Cmpd. II
5 uM
0.5 uM
50 nM
5 nM
TGFb
BSA

Figure 21B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/18951

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 31/535, 31/505, 31/44, 31/47, 31/415
US CL :514/235.8, 275, 306, 314, 341, 394, 397
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/235.8, 275, 306, 314, 341, 394, 397

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,206,258 A (DORIA et al.) 27 April 1993.	1-14
A	US 5,246,699 A (DEBRE et al.) 21 September 1993.	1-14
A,P	US 5,656,644 A (ADAMS et al.) 12 August 1997.	1-14
A,P	US 5,658,903 A (ADAMS et al.) 19 August 1997.	1-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 30 DECEMBER 1997	Date of mailing of the international search report 29 JAN 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer RAYMOND J. HENLEY III Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*